USE OF THE PUPAL SURVEY TECHNIQUE FOR MEASURING Aedes aegypti (DIPTERA: CULICIDAE) PRODUCTIVITY IN PUERTO RICO

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Abstract. The hypothesis tested was that most pupae of Aedes aegypti are produced in a few types of containers so that vector control efforts could concentrate on eliminating the most productive ones and thus prevent dengue outbreaks. Pupal surveys were conducted twice in 2004 in an urban area in southern Puerto Rico. A total 35,030 immature mosquitoes (III and IV instars, pupae) was counted in 1,367 containers found with water in 624 premises during the first survey. Only pupae were counted in the second survey in 829 premises, 257 of which had containers with water, and 124 contained Ae. aegypti pupae (15%, 22% in the first survey). We found fewer (583) containers with water than in the first survey, but 202 had pupae (35%; 18.5% in first survey). Containers yielded 3,189 Ae. aegypti pupae, which was slightly fewer than those found in the first survey (3,388 pupae). The hypothesis was supported by the data, showing that 7 of 18 types of containers contained 80% of all female pupae. The most productive containers generally were also common. We used several criteria (i.e., container use, two-step cluster analysis based on environmental variables of containers and premises) to classify the containers and premises and to evaluate pupal distribution at various spatial scales (container, premise, and residences versus public areas). Most pupae were in 4 of 10 types of container usage categories. The cluster technique showed that most pupae were in unattended, rain-filled containers in the yards, particularly in receptacles in the shade of trees that received rainfall through foliage and had lower water temperatures. Pupal counts were adjusted to a negative binomial distribution, confirming their highly aggregated dispersal pattern. Cluster analysis showed that 61.3% of female pupae were in 40 (6.4%) of 624 premises that had in common their larger yards, number of trees, and container water volume. Using number of Ae. aegypti larvae, Breteau Index, or the presence of immature forms as indicators of pupal productivity is not as efficient in identifying the most productive types of containers as direct pupal counts.

INTRODUCTION

As part of a multinational initiative stimulated by the Special Program for Research and Training in Tropical Diseases (United Nations Children’s Fund/United Nations Development Program/World Bank/World Health Organization [UNICEF/UNDP/World Bank/WHO]) to evaluate the generalizability of the pupal survey technique to monitor Aedes aegypti productivity,1,2 we investigated the applicability of this methodology in an urban community in Puerto Rico. The pupal survey technique generates an estimate of pupal density of Ae. aegypti in containers as a proxy for the number of adults. The number of females emerging daily under steady-state conditions is the product of the number of pupae present in the area, the proportion of pupae emerging every day (inverse of the pupal development time), the proportion of female pupae, and the pupal survival rate. The standing crop (adults of all ages) is calculated by multiplying daily female emergence times the life expectancy of the female adult.3 The hypothetical minimum number of females Ae. aegypti required to cause a dengue outbreak (threshold density; female pupae per person) has been modeled based on environmental temperature, rate of virus introductions, and human population immunity. Contrasting the actual density of Ae. aegypti with the corresponding threshold gives an idea of the magnitude of vector population reduction that would be required to prevent dengue outbreaks.4 Although the concept of vector population threshold for dengue awaits field validation, using pupae as a proxy for the number of adults seems well-supported. Issues about practicality in vector control programs and reliability of pupal surveys are being investigated as part of the Special Program for Research and Training in Tropical Diseases multinational research project.

The pupal survey may be a feasible approach for vector control programs because preliminary observations in several urban areas suggested that most pupae of Ae. aegypti were produced in a few types of containers.3 Thus, vector control efforts can be concentrated on eliminating or treating the most productive types of containers to reduce mosquito density below a target threshold. Container abundance and productivity determine pupal standing crop, and it is thought that after identifying the most productive container types in a given area, further surveillance will only be needed for assessing the abundance of each container type. In other words, counting pupae would not be needed every time the same area is surveyed.2

We explored and expanded the following aspects of the hypothesis that most pupae of Ae. aegypti could be located in specific portions of the urban habitat. 1) Most pupae of Ae. aegypti are produced in a few types of containers, regardless of the way containers are grouped in classes (e.g., container name, householder’s container usage). 2) Most pupae of Ae. aegypti are found in a few containers of each type (aggregation) whereas most containers do not have pupae. 3) The most productive container types are the most abundant. 4) Most pupae of Ae. aegypti are found in a few key premises.4 5) There are significant differences in Ae. aegypti productivity between public areas (streets, parks, empty lots, etc.) and households. 6) Environmental conditions of containers and premises determine pupal productivity, and those conditions can be used as indicators of pupal productivity. 7) The most productive container types vary in time. 8) Assessing number of larvae per type of container, percentage of positive containers of each class, or Breteau Index per type of container is as efficient in detecting which types of containers have high pupal productivity as direct pupal counts.

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MATERIALS AND METHODS

The study was conducted from May to July and October to December 2004 in Community Playa–Playita, municipality of Salinas in southern Puerto Rico (17°58′N, 66°18′W; Figure 1). Mean annual temperature and annual rainfall from 1990 through 2003 were 26.6°C and 973 mm, respectively. The hot, rainy season is from May to November and the drier and cooler season is from December to April (Figure 2). Vegetation surrounding the town was mainly agricultural fields, dry forests, and mangroves. The town had 1,344 premises with structures that were characterized as households (84.0%), abandoned buildings (9.9%), stores (4.7%), schools (0.9%), and churches (0.5%). There were 3.04–3.15 persons/household (U.S. Census Bureau 2000), or the equivalent of 3,500 inhabitants. Streets and buildings were digitized and incorporated into a geographic information system (GIS) (TNTmips, Microimages, Inc., Lincoln, NE and ArcGis, Environmental Sciences Research Institute, Inc., Redlands, CA) from a scanned aerial photograph (Puerto Rico Transportation Authority, October 23, 2000, 1:20,000), a map (Puerto Rico Revenue Service), and from field surveys with global positioning units (Garmin Rino 120; Garmin International, Inc., Olathe, KS). The GIS assigned a unique identifier to each structure that was used to randomly sample premises (Programs for Ecological Methodology), where we looked for containers with water and immature mosquitoes. All streets and empty lots were visited to sample containers with water and mosquitoes. We did not search for containers inside buildings because previous observations in Puerto Rico indicated that most containers with water were outdoors.

We recognized 18 different types of containers in the study area and 10 different householder’s uses of the observed containers. Within the classification by type (Figure 3), we recognized five classes of discarded containers: discarded (once used to preserve or store goods, such as bottles, cans, etc.), discarded implements (damaged appliances, such as sinks, toilet seats, etc.), discarded kitchen utensils (dishes, pots, pans), other discarded utensils (coolers, fish bowls, etc.), and discarded or damaged furniture. Large and small buckets were 5 and 2.5 gallons, respectively. Drums were large plastic or metal vessels (55 gallons). Covers were plastic sheets used to protect objects from the rain. Plant pots included the pot itself and a dish under the pot that is used to contain excess water.
Classes of container usage (Figure 4) grouped containers regardless of their type. For example, a broken plastic sheet cover was a discarded container; a bucket could be used as an animal drinking pan, etc. Ornamental containers included plant pots and water fountains. Cleaning and object storage containers were mainly buckets and other devices for this purpose. Water storage containers were mainly drums, tanks, and other large receptacles. Recreational containers were boats, plastic pools, grills, etc. Household-repair containers were trays, vessels used to mix construction materials, etc. Discarded containers were all vessels thrown in the backyard without being used, including tires.

The following data were recorded for each container with water during the first survey: container name, container use, water volume (mL), water temperature (°C), presence of early instars (I and II), number of advanced instars (III and IV), and number of male and female pupae. We also noted the source of water in containers (i.e., human placement, direct rainfall, rain through foliage, rain off the roof), degree of exposure to the sun (e.g., full, partial, shade), container material (e.g., plastic, metal, porcelain, glass, cement/clay, rubber, cloth/paper, plant material), presence of tree canopy, and the tree species over the container (stems with a diameter > 5 cm at chest height). During the second survey, we recorded the same variables except the number of larvae, water volume, and water temperature. Plant identifications were made using the classifications of Little and others.7,8 For each premise, we recorded its area, the area of the structure, and tree abundance and species. Larvae in early instars (I and II) were taken to the laboratory where they were reared to the fourth instar for identification purposes. Pupae were placed in petri dishes with a damp filter paper at the bottom, transported to the laboratory, and placed in cages for emergence, identification, and weighing. Dead pupae were preserved in 80% ethanol and identified to species. Emerging adults were killed in a freezer (−20°C) and oven-dried (45°C for 2 days). Individual mosquitoes were weighed using a Thermo Cahn C35 microbalance (Thermo Electron Corporation, San Jose, CA) (1/ug). Mosquito identifications were made using published information.9–14

Statistical analyses. Summary values were expressed as the mean ± SE. Paired t-tests were conducted to test for mean differences in the number of males and females per container, and to compare female pupae of Ae. aegypti per premise that were found in the same premises in the two surveys. The null hypothesis that numbers of female pupae of Ae. aegypti did not differ among container types, container use, or among derived clusters was evaluated using Kruskal-Wallis analyses. Mean dry mass of emerging adults was compared among categories of container type, container use, or clusters by means of one-way analysis of variance (ANOVA).

The hypothesis that a few containers within each type had most of the pupae was evaluated by analyzing their variance/mean ratio, which is expected to be less than 1 for a uniform distribution, 1 for a random distribution, and more than 1 for an aggregated distribution (chi-square index of dispersion test).15 The null hypotheses stating that female pupae had a Poisson distribution (variance/mean = 1) or a negative bino-

Figure 2. Mean annual rainfall (mm) and mean temperature (°C) of the study site in Community Playa–Playita, municipality of Salinas, southern Puerto Rico, 1990–2003.
FIGURE 3. Distribution of female pupae (top) and total number of vessels (bottom) per type of container in Salinas, Puerto Rico, May–July 2004.
mial distribution (variance/mean > 1) were evaluated (chi-square goodness-of-fit test)\textsuperscript{15} for each type of container.

A two-step cluster analysis was used to classify containers with water based on the following variables: container use, source of container water, presence or absence of trees above the container, amount of exposure to the sun, container material, and water volume (transformed to four ranks with similar sample size: < 0.130, 0.140–0.490, 0.500–1.800, and > 1.800 liters). Two-step cluster analysis can show natural groupings or clusters, handle categoric and continuous variables, and automatically select the number of clusters (model) by means of information criteria (Bayesian Information Criterion). Once the groups were identified, we calculated statistics of female pupae per container for each group, and conducted a one-way ANOVA to compare mean pupae across groups.

A two-step cluster analysis was conducted to explore the existence of homogeneous groups of structures with \textit{Ae. aegypti}. The variables included in the classification were the area of the premise (m\textsuperscript{2}), mean water volume (liters) in con-

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure4.png}
\caption{Distribution of female pupae (\textit{top}) and total number of vessels (\textit{bottom}) per class of container in Salinas, Puerto Rico, May–July 2004.}
\end{figure}
tainers on the premise, mean number of trees, whether the building was inhabited or vacant, type of structure as indicated earlier in this report, and total *Aedes aegypti* female pupae per premise. In this case, we did not make statistical comparisons among groups because we included the number of pupae in the classification criteria. To evaluate if premises changed their status as producers (with pupae) or non-producers (without pupae) between surveys, we compared the premises sampled on both occasions (505 premises) using a $2 \times 2$ contingency table (pupae present/absent, 2 surveys) and a chi-square test (degrees of freedom [df] = 1).

**RESULTS**

A total of 35,030 immature mosquitoes (III and IV instars and pupae) was counted in 1,367 containers found with water in 624 premises studied during the first pupal survey (May–July 2004). The immature stages of the following mosquito species were found in containers in the patios or gardens of premises: *Ae. (Stegomyia) aegypti* (L.), *Ae. (Gymnometopa) mediovittatus* (Coquillet), *Anopheles grabhamii* Theobald, *Culex bahamensis* Dyar and Knab, *Cx. habilator* Dyar and Knab, *Cx. janitor* Dyar and Knab, *Cx. nigripalpus* Theobald, *Cx. quinquefasciatus* Say, and *Culex* (*Melanocoenon*) sp. Larvae of *Cx. nigripalpus* and *Cx. habilator* could not easily be differentiated based on current taxonomic descriptions. Relative abundance of mosquitoes was *Ae. aegypti* (58.0% of total individuals), *Cx. quinquefasciatus* (28.0%), other *Culex* spp. (13.4%), and *Ae. mediovittatus* (0.6%). *Anopheles grabhamii* and *Cx. bahamensis* were rarely collected (one larva each). A few (13) sites (e.g., discarded tires, ground pools) were used with water and mosquitoes in public areas (*Ae. aegypti*, *Ae. mediovittatus*, *An*. *grabhamii*, *Cx. quinquefasciatus*, *Cx. nigripalpus*, *Cx. (Mel.) atratus* Theobald, and *Cx. (Mel.) isoldomis* Dyar). Because only 321 immature mosquitoes were found in public areas, the rest of the analysis concentrated on containers sampled in patios and gardens of residential premises. Traditional *Stegomyia* indices from the first survey were a Premise or House Index of 29.2% (182 positive premises of 624 sampled), a Container Index of 28.8% (percentage of containers with water and *Ae. aegypti*), and a Breteau Index of 62 (number of positive containers per 100 premises inspected). *Aedes aegypti* pupae (3,388; 1,826 males and 1,562 females) were present in 246 (18.0%) of 1,367 containers with water. Male *Ae. aegypti* pupae per container (mean ± SE = 1.34 ± 0.19) were more abundant than female pupae (1.14 ± 0.15; $t = 2.23$, df = 1,366, $P < 0.05$, by two-tailed paired $t$-test).

*Aedes aegypti* pupae per type of container. Seven types of containers (large and small buckets, plastic sheets used to cover large objects, discarded and implements, toys, and drums; Figure 3) yielded 80.5% of all pupae and represented approximately half (53.1%; Figure 3) of all containers surveyed. The null hypothesis stating that female pupae of *Ae. aegypti* did not differ among container types was rejected ($\chi^2 = 60$, df = 9, $P < 0.01$, by Kruskal-Wallis analysis). A Spearman correlation between the rank orders of container abundance and pupal density was positive and significant ($n = 18; r_s = 0.69, P < 0.01$) suggesting that the most productive containers were also common. The correlation was far from perfect because some unusual containers had comparatively high pupal productivity (e.g., plastic sheets used as covers and toys; Figure 3). The seven most common types of containers had 60.4% of all pupae, which reflected the positive correlation between container abundance and productivity.

Mean individual dry mass of emerging *Ae. aegypti* males did not differ among the five types of most productive containers (range = 0.233–0.351 mg; $F_{4,114} = 2.18$, $P > 0.05$, by ANOVA). Mean individual dry mass of emerging *Ae. aegypti* females differed significantly among those five container types (range = 0.377–0.612 mg; $F_{4,114} = 2.49$, $P < 0.05$, by ANOVA). The largest females came from pockets of rain water accumulated on plastic sheets that were used to cover large objects on backyards or from discarded cover sheets. The total biomass per type of container (female pupae × average body mass) was larger for cover sheets (135.8 mg), followed in order by large buckets (122.9 mg), discarded containers (75.8 mg), small buckets (65.8 mg), and discarded utensils (59.4 mg).

The hypothesis that a few containers within each type had most of the pupae was evaluated by analyzing their statistical distributions and variance: mean ratios per type of container. The null hypothesis of a Poisson (random) distribution (variance/mean = 1) was evaluated (chi-square goodness-of-fit test) for each type of container. We concluded that the variance/mean ratio was significantly different from 1 ($P < 0.01$) for each type of container (variance/mean ratios = 3.4–62.9), and by inspecting the distributions and the ratios, it was concluded that female pupae had a clumped distribution. Container types with few samples were not evaluated. The distribution of female pupae per type of container was fitted to a negative binomial distribution (chi-square goodness-of-fit test) for discarded utensils, small buckets, discarded containers, other utensils, large buckets, tires, and plant pots. The null hypothesis that female pupae of *Ae. aegypti* had a negative binomial distribution could not be rejected ($P > 0.05$) for any of the container types studied. Aggregation of female pupae was evident, for example, since only 29 (24%) of 122 discarded implements contained pupae, and among those that were positive only 12 (10%) had approximately 80% of the pupae. The mean ± SE percentage of containers having 80–90% of all pupae across the 11 most abundant types was 6.9 ± 1.3%, or 94 (7%) of 1,367 containers with water in the study area.

Alternate ways of classifying the containers. Given the diversity of container types, we classified them by use or function in the household, thus reducing the number of classes to 10 (Figure 4). Collectively, the most numerous containers were discarded containers, those used for animal drinking, ornamental purposes, and household cleaning (90%; Figure 4). Discarded containers were the ones that produced most of the female pupae (44.6%). Discarded containers, ornamental vessels, cover sheets, and toys accounted for 77.2% of all pupae (Figure 4). The null hypothesis that numbers of *Ae. aegypti* female pupae did not differ among classes of container usage was rejected ($\chi^2 = 47.4$, df = 9, $P < 0.01$, by Kruskal-Wallis analysis). There was no significant correlation between the rank orders of container and pupal abundance ($n = 10; r_s = 0.39, P > 0.05$) mainly because the second most abundant category of container use (animal drinking pans) did not produce many pupae (Figure 4). However, the four most abundant containers classified by use (discarded, orna-
mental, cleaning, and animal drinking) had 69.6% of all pupae.

The distribution of female pupae per category of container usage was also clumped, with significant variance: mean ratios (2.8–137.7) for each container usage. A negative binomial distribution could be fitted to discarded, ornamental, and cleaning containers but not to animal drinking and water storage containers. Aggregation was evident in the discarded containers. For example, female pupae were present in 114 (16.6%) of 686 discarded containers, but only 41 (6%) of all discarded containers contained 80% of the pupae.

Mean individual dry mass of emerging *Ae. aegypti* males did not differ among the four categories of container use containing most of the pupae (range = 0.266–0.339 mg; F₃,₁₅₇ = 0.63, P > 0.05, by ANOVA). Mean individual dry mass of emerging *Ae. aegypti* females significantly differed among those container types (range = 0.421–0.600 mg; F₃,₁₄₈ = 3.34, P < 0.05, by ANOVA). The largest females came from pockets of rain water accumulated on plastic sheets that were used to cover large objects in backyards. Total biomass per type of container (female pupae × average body mass) was larger for discarded objects (272.3 mg), followed by covers (121.2 mg), cleaning utensils (57.7 mg), and ornamental containers (56.7 mg). The rank order of standing crop per category of container use was essentially the same as that for total number of female pupae.

**Cluster analysis of containers.** The two-step cluster analysis produced four groups of containers (I–IV; Table 1). Cluster I (26.2% of samples) comprised most of the containers used for animal drinking, ornamental vessels, and containers used for cleaning (Figure 5). Cluster I had 0.5 ± 0.2 female *Ae. aegypti* pupae per container, which represented 10.1% of all female pupae (Table 1 and Figure 5). Cluster II included 12.5% of all containers, mostly ornamental receptacles and water storage containers (Figure 5). This group had the largest mean ± SE water volume per container (19.7 ± 4.9 L) of the four clusters (Table 1). Cluster II had 1.3 ± 0.4 female pupae per container and 13.9% of all female pupae. Clusters III (32.6% of all containers) and IV (28.7%) included 96.4% of the discarded containers (Figure 5). Most containers in cluster III were exposed to the sun with the highest mean water temperature, no tree canopy, and received direct rainfall or rain draining off the roof (Table 1). In contrast, containers in Cluster IV were mostly in the shade of trees, received rainfall through foliage, and had the lowest mean water temperature (Table 1). Clusters III and IV had 26.6% and 49.4%, respectively, of all female pupae, and their female pupal densities were 1.0 ± 0.2 and 2.0 ± 0.3, respectively. It is readily appreciated (Figure 5) that some uncommon containers had comparatively large numbers of *Ae. aegypti* pupae (cover sheets and toys), and that pupal productivity was higher when containers were under the environmental conditions of cluster IV (Table 1).

Also, as previously indicated, some rather common containers (animal drinking) had comparatively low productivity (Figure 5). A one-way ANOVA (F₃,₁₁₅₉ = 5.95, P < 0.01) showed significant differences in the mean density of female *Ae. aegypti* pupae among clusters. Most containers in Clusters I and II were those frequently attended by humans in the process of sustaining plants and animals on their premises (24% of total female pupae), whereas those in Clusters III and IV were basically unattended and rain-filled (76% of total female pupae).

Mean individual dry mass of emerging *Ae. aegypti* males (range = 0.261–0.329 mg; F₃,₁₇₇ = 1.01, P > 0.05, by ANOVA) or females (range = 0.3721–0.445 mg; F₃,₁₇₀ = 0.72, P > 0.05, by ANOVA) did not differ among the four clusters. Conversely, total biomass (female pupae × average body mass) was largest for cluster IV (313.3 mg), followed in decreasing order by cluster III (155.9 mg), cluster II (75.1 mg), and cluster I (65.4 mg). The percentage of total female biomass produced in unattended, rain-filled containers (76.8% in clusters III and IV) was similar to the percentage (76%) of all female pupae produced in those containers.

**Variability in pupal production among premises.** Mean ± SE female pupae of *Ae. aegypti* per type of structure (percentage of total pupae) were uninhabited house = 2.8 ± 1.9 (14.7%), inhabited house = 2.4 ± 0.4 (78.9%), abandoned building = 1.9 ± 0.9 (5%), store 0.7 ± 0.3 (1.4%), and church = 0. Three clusters were derived. The first cluster (103 premises) included abandoned structures (100%) and uninhabited houses (94.5%). Cluster II (76 premises) contained all commercial stores, two of the three churches, four uninhabited houses (5.5%), and a few inhabited houses (9.2%). Cluster III (396 premises) included only most of the inhabited houses (94.5%; Table 2). Most female pupae of *Ae. aegypti* were present in premises of cluster II (62.3%). This group had large premises, a large number of trees, and container water volumes (Table 2). Within cluster II, the 40 inhabited and 4 uninhabited premises yielded 97.6% of all *Ae. aegypti* female pupae in this cluster, which indicated that buildings used for commercial purposes (stores, and hotels) and churches were grouped in cluster II basically because of their large lot sizes, but otherwise contributed little to *Ae. aegypti* mosquito productivity. Most containers with water were in cluster III (inhabited premises; Figure 6); however, a large proportion of all the female pupae were in cluster II (very few premises) in a few classes of uncommon (plastic cover sheets, toys, drums) and common (large buckets, discarded) highly productive

![Table 1](image-url)
containers (Figure 6). For example, there were many more discarded containers and large buckets in the premises of cluster III than in cluster II, but the total number of female pupae was similar in discarded containers and larger in the large buckets of cluster II (Figure 6). It can also be seen that the highly productive, uncommon containers were producing pupae almost only in premises of cluster II (covers, toys, drums; Figure 6). Therefore, a few premises were highly productive because of the presence of those unusually productive vessels and a global higher productivity in containers in premises of cluster II, which was associated with larger lots, numerous trees, and larger container water contents.

**Figure 5.** Female pupae of *Aedes aegypti* (top) and total number of vessels (bottom) found in each type of container use in Salinas, Puerto Rico, May–July 2004 for each of four groups of containers classified using a two-step cluster analysis based on environmental variables.
Temporal variation in Ae. aegypti female pupae. The second survey in Salinas (October–December 2004) included 829 premises with 257 containers with water, and 124 premises had Ae. aegypti pupae (15%; 22% in the first survey). We found fewer (583) containers with water (1,367 in 1S), but 202 of them had pupae (35%; 18.5% in the first survey). Containers had a total of 3,189 Ae. aegypti pupae (1,731 males and 1,458 females), which was slightly fewer than those found in the first survey (3,388 pupae). Male Ae. aegypti pupae per container (2.97 ± 0.44) were again more abundant than females (2.50 ± 0.38; t = 2.37, df = 582, P < 0.05, by paired t-test). The number of female Ae. aegypti pupae per container in the second survey was significantly larger (t = 2.71, df = 953, P < 0.01, by unequal variance) than in the first survey (1.14 ± 0.15). Therefore, during the second survey, we found fewer containers with water and pupae, but a higher pupal density per container than in the first survey. At the premise level, there were no statistical differences in the mean number of female pupae per premise (t = 1.11, df = 1,446, P > 0.05) between the first (2.34 ± 0.38) and second (1.76 ± 0.36) surveys.

To evaluate if premises changed their status from producers (with pupae) to non-producers (without pupae) between surveys, we compared the 505 premises sampled in both occasions using a 2 × 2 contingency table (pupae present/absent surveys). The test result was significant (χ² = 14.7, df = 1, P < 0.01), indicating changes in the status of premises as producers of Ae. aegypti between the two survey periods. For example, 71 premises that produced pupae in the first survey did not have pupae in the second one, whereas 51 premises that were negative in the first survey had pupae during the second sampling. Continued production occurred in 29 premises, but most houses (351) were negative in both periods.

The container types with most of female pupae of Ae. aegypti varied in rank in the second survey, as shown by the lack of statistical significance of the Spearman’s rank correlation coefficient (n = 17; rₛ = 0.44, P > 0.05) between total pupae produced by each type of containers in each period (Figure 3). The six container types with most female pupae (78.8%) were large buckets, discarded containers, small buckets, tires, other discarded utensils, and plastic pools (Figure 3). The correlation between rank orders of container abundance and total pupal per container type in the second survey was significant (n = 18; rₛ = 0.88, P < 0.01). Targeting the six most common containers would bring about a similar (73%) reduction in pupal production as targeting the six most productive containers. Common, most productive container types in both surveys were small and large buckets and various types of discarded containers (Figure 3).

Grouping containers by their use showed that 71.3% of all female pupae were in only one category of containers (discarded ones; Figure 3). Some uncommon containers, such as the 4 plastic swimming pools had a large number of female pupae, but all 113 pupae were found in only 1 pool. Ornamental containers (plant pots and fountains) and objects used for cleaning purposes (buckets) were important in both sampling periods (Figure 3). The correlation between rank orders of container and pupal abundance was not significant (n = 10; rₛ = 0.55, P > 0.05), as in the first sampling period for container usage, which reflected the importance of scarce containers (plastic pools) with a disproportionate number of female pupae (Figure 5). Therefore, in both surveys we found at least one category of scarce container-usage that was producing a large number of pupae (plastic cover sheets and pools). It is also noteworthy that plastic covers, toys, and water storage containers exhibited rather low pupal productivity in the second survey.

Larval Breteau, and positive-container counts as indicators of what types of containers had the most pupae of Ae. aegypti. Ranks of larval abundance per type of container correlated with the order of female pupal abundance per type of container (n = 18; rₛ = 0.59; P < 0.01). Although 8 (44.4%) of 18 types of containers had 81.8% of the larvae and 70.9% of the female pupae, it was necessary to add the pupae in 11 types of containers with the largest number of larvae to account for 81.1% of the female pupae. In other words, 11 types of containers would have to be eliminated to achieve 81.1% control of female pupae based on larval counts, whereas direct counts of pupae that identified the most productive containers showed that eliminating 7–8 types of containers would bring about a reduction of 79.7–85.2%. Similarly, using the Breteau Indices per container type, we observed that 11 types of containers accounted for 81% of the female pupae. The ranks of percentage of positive containers did not correlate with pupal abundance ranks per type of container (n = 18; rₛ = −0.02; P > 0.05).

Using larval counts per class of container usage, 7 of 10 classes of containers had 80.1% of the pupae, compared with 4 classes of containers with 80.3% of the pupae if pupal counts were used. There were no correlations between larval abundance (n = 10; rₛ = 0.33; P > 0.05) or percentage of positive containers (n = 10; rₛ = 0.32; P > 0.05) and pupal abundance per class of container usage.

DISCUSSION

The hypothesis that most pupae of Ae. aegypti are produced in a few types of containers² was supported by the data

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<th>Derived</th>
<th>No. of buildings/ % of total</th>
<th>No. of buildings</th>
<th>Area of premise (m²)</th>
<th>Mean water volume in containers (L)</th>
<th>Mean no. of trees per premise</th>
<th>Total female pupae of Ae. aegypti / % of total pupae</th>
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<tbody>
<tr>
<td>I</td>
<td>103 (17.9)</td>
<td>0</td>
<td>444 ± 31</td>
<td>2.6 ± 0.9</td>
<td>6.8 ± 0.7</td>
<td>132 (9.2)</td>
</tr>
<tr>
<td>II</td>
<td>76 (13.2)</td>
<td>60</td>
<td>853 ± 106</td>
<td>41.3 ± 9.6</td>
<td>19.9 ± 3.5</td>
<td>892 (62.3)</td>
</tr>
<tr>
<td>III</td>
<td>396 (68.9)</td>
<td>396</td>
<td>487 ± 14.2</td>
<td>3.4 ± 0.4</td>
<td>10.0 ± 0.5</td>
<td>407 (28.4)</td>
</tr>
<tr>
<td>Total</td>
<td>575</td>
<td>456</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1431</td>
</tr>
</tbody>
</table>

* Most buildings in cluster I were abandoned structures, uninhabited houses, stores, and churches. Most buildings in cluster II and III were inhabited houses.

**Table 2:** Two-step cluster analysis of premises in Salinas, Puerto Rico (May–July 2004) and statistics (mean ± SE) of the variables used in the classification process
Figure 6. Female pupae of *Aedes aegypti* (top) and total number of vessels (bottom) found in each type of container in Salinas, Puerto Rico, May–July 2004 for each of three groups of premises classified using a two-step cluster analysis based on environmental variables.
collected in Salinas. For this locality, between 6 and 7 of 18 types of containers had 73–80% of all female pupae. The order in pupal productivity per type of container did not remain the same in the two surveys, but buckets and discarded containers, utensils, and implements were consistently large producers. Two types of infrequent but highly productive containers (plastic covers and plastic pools) shifted in importance as pupal producers between surveys (Figure 3). We used a container-type classification with 18 classes, but the number of different container classes could have been much larger. For example, within the discarded implement class, we found a variety of once useful but now discarded objects, such as baby seats, different types of trays from old appliances, diverse auto parts, sinks, toilet seats, fish bowls, suitcases, iron machines, and chairs, among other objects. Therefore, our conclusions were reached in reference to the classification that we used, which was chosen to not be excessively large. Our recommendation about this sampling limitation is to be consistent and use the same classification every time because there is no single, universal way to classify the potential containers where Ae. aegypti could develop in urban areas.

The hypothesis was also supported when the containers were classified by the use, function, or role that householders gave the containers (Figure 4); in this case the total number of container uses that we identified was 10. During the first survey, four types of container use (discarded containers, plastic cover sheets, ornamental containers, and toys) had 80.3% of all pupae, while during the second survey a single class of container usage (discarded containers) produced 71.3% of all pupae. Therefore, controlling or eliminating Ae. aegypti immature forms in receptacles that have been discarded or used as covers, ornamental containers (plant pots and water fountains), or toys could substantially reduce the immature population. A rather abundant class of container use, animal drinking vessels, contained only 4.5–5.8% of all pupae. Similarly, the infrequent water storage vessels in our study area held only 1.4–4.3% of all pupae. It appears that classifying the containers by household use or function gave more parsimonious results and ease of interpretation than the classification by type or name (Figures 3 and 4).

Additionally, we explored whether the most productive container types corresponded with the most abundant containers in the study area because in such a case it would not be necessary to target the most productive ones (with the associated costs of counting pupae). This hypothesis was not always supported by the data because a few types of scarce containers had large numbers of pupae, or because some rather common ones, such as the animal drinking pans, had few pupae. Reductions of pupae of 60–80% could be accomplished by controlling the most common containers, but this would involve a larger number of total containers to be controlled compared with an understanding of pupal productivity. In practice, the costs of eliminating Ae. aegypti from some types of containers may significantly vary. For example, it may be more difficult to control immature forms in animal drinking pans than in containers that are possibly not as sensitive for householders. In this study, the low productivity observed in animal drinking pans may not justly target them for mosquito control, but this was a result of analyzing pupal productivity. Therefore, our recommendation is to understand local pupal productivity per type of container, then to target containers based on their abundance and productivity maximizing the impact on the mosquito population and minimizing operational costs and time.

The quantitative approach followed here to classify the containers (two-step cluster analysis) based on container variables (use of the container, source of water, presence of tree canopy, degree of exposure to the sun, type of container material, and water volume) showed that most (80%) pupae found in the study area were in unattended, rain-filled containers in yards. The most productive containers were those in the shade of trees that received rainfall through foliage and had lower water temperatures. The rest of pupae were in containers used for the residents mainly to sustain animals or plants or to store water (animal drinking pans, plant pots, ornamental fountains, barrels, buckets, etc.; Figure 5). From this classification system, we concluded that a significant reduction in the Ae. aegypti population could be achieved by household management (removing discarded containers and placing essential receptacles under a roof or upside down) of their backyards in Salinas, without necessarily having to deal with those containers to which water is added by people.

Using the same multivariate technique, we classified the premises in the study area using several structural variables of the premises (surface area, number of trees, whether or not the building was inhabited, type of building) and containers (volume of water, total female pupae). A single 76 premises, a new class of container use (animal drinking pans, plant pots, ornamental fountains, barrels, etc.) contained 62% of all female pupae (Table 2). Those premises were characterized by having the largest surface areas, number of trees, and containers with large volumes of water. Recognizing the existence of a reduced number of premises with such large pupal yields is an important additional criterion to guide eventual control efforts. Those premises were the ones containing the uncommon (plastic cover sheets, toys) or common (discarded, large buckets) highly productive vessels. It has been observed in Queensland, Australia that a small percentage of premises contained a large percentage of positive containers and that poorly-maintained houses with untidy, shaded yards were 2.5 times more likely to be positive for Ae. aegypti immature forms (Premise Condition Index). The Premise Condition Index was significantly associated with the percentage of positive premises and the number of positive containers per premise in Colima, Mexico. Two of the three qualitative variables that make up the Premise Condition Index are yard conditions (trash and lawn maintenance) and tree shade conditions. Therefore, our results agree with previous observations that premises with more trees and an accumulation of discarded containers in the yards produce more Ae. aegypti.

With one exception, the individual biomass of emerging Ae. aegypti adults did not differ between types of containers. Female adults emerging from plastic cover sheets were significantly heavier than in other types of containers. This result underscores the importance of some uncommon containers, which could be overlooked in surveillance and control programs. The rank order of pupal standing crop (pupal density × individual weight) was similar to the rank order of the total number of pupae per type of container use and per clusters of containers.

The results presented are consistent with a general theme: that most pupae are present in a small fraction of all containers or premises. This pattern is usually characterized by sta-
tistical distributions that take into account over-dispersal (aggregation and contagion), where most samples are empty and a few samples contain most of the individuals. The hypothesis that regardless of the type or category of containers only a few receptacles within each class had most of the individuals was supported by the data collected in this study. The distribution of female pupae per container for the most abundant containers was adjusted to a negative binomial distribution. The parameter \( k \) of the negative binomial was low and the variance:mean ratio was large, showing that \( Ae. aegypti \) immature forms were highly aggregated in the containers. Evidence suggests that larva of \( Ae. aegypti \) were not just aggregated but probably overcrowded. We have observed that the number of larvae and pupae of \( Ae. aegypti \) per container in Salinas were positively correlated, but the body mass of emerging females was negatively correlated with larval density (Barrera R. and others, unpublished data). Concurrent observations led us to conclude that food limitation or intraspecific competition were limiting factors for \( Ae. aegypti \) in a large fraction of the containers in the study area (Barrera R. and others, unpublished data). Conversely, lack of negative density-dependent effects of larvae on emerging adults seemed to exist in a smaller fraction of the containers with larger volumes of water and lower temperatures.

A similar pattern of aggregation was demonstrated for the number of female pupae per premise. Reuben and others\(^{18}\) found that the pupal population of \( Ae. aegypti \) per premise in Sonapat, India followed a negative binomial distribution, with values of the aggregation parameter (\( k \)) ranging from 0.021 to 0.095 in several surveys of the same area in time. The \( k \) values for female pupae per premise in the present study were 0.058 and 0.038 for the first and second surveys, respectively. With some exceptions, aggregation of immature forms and pupae has been commonly observed in a number of mosquito species.\(^{19}\)

Aggregation was observed in both surveys, in spite of the temporal changes observed in the relative abundance of the most productive containers and the mean number of pupae per container. Aggregation is largely a result of lack of individuals in most samples. For example, of 505 premises sampled in both periods, 351 (70\%) had no pupae at the time of the survey either because those premises did not have containers with water or because the containers had no pupae. Conditions associated with pupal production changed in premises over time because 14\% of the premises with pupae in the first survey did not have pupae in the second survey, and 10\% of the premises without pupae in the first survey period did have pupae in the second survey. These results and the observed changes in the abundance of the most productive containers between surveys emphasize that these aquatic habitats are dynamic and the need for frequent assessments of premises.

The use of counts of developed larvae (third and fourth instars), the presence or absence of immature forms, or the Breteau Index did not substantially improve our perception of which and how many classes of containers had most female pupae (roughly 80\%), compared with direct determination of the most productive containers by counting pupae. Using pupal counts, control efforts could concentrate in the elimination of fewer classes of containers to achieve a relatively large reduction in the production of adults. The pupal survey approach would not be of much use if pre-adult control will be applied to every type of container in the study area. Therefore, the pupal survey is a precise way to stratify the environment with the purpose of guiding and making the control of pre-adult \( Ae. aegypti \) more effective and economical.

From the various approaches we followed here to understand pupal productivity, we conclude that a significant reduction in the \( Ae. aegypti \) population could be achieved by household management of their backyards in Salinas (removing discarded containers and placing essential receptacles under a roof or upside down), without necessarily having to deal with those containers to which water is added by people. It was also evident in Salinas that inhabited premises with larger lot sizes and more trees are likely to produce more pupae, thus providing the means to prioritize areas for rapid abatement of much of the population of immature forms. The usefulness of the pupal survey approach, with varying composition of aquatic habitats of \( Ae. aegypti \), is to be reported by the multinational research group financed by the Special Program for Research and Training in Tropical Diseases (TDR; UNICEF/UNDP/World Bank/WHO), who is evaluating the generalizability of this technique in several countries.

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