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**A
Review
of
Entomological
Sampling Methods
and Indicators for
Dengue Vectors**



**A REVIEW OF ENTOMOLOGICAL SAMPLING METHODS
AND INDICATORS FOR DENGUE VECTORS**

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Summary

This review was developed in response to a recommendation of the WHO Informal Consultation on Strengthening Implementation of the Global Strategy for Dengue Fever/Dengue Haemorrhagic Fever Prevention and Control, held in October of 1999, urging “the refinement of existing entomological indicators and/or the development of new indicators that better reflect transmission potential.” The Consultation “recommended that such indicators should provide clear, meaningful information for communities as well as for programme managers and policy-makers.” Whereas the traditional *Stegomyia* indices (the House, Container, and Breteau indices, and various related derivations) are of some operational value for measuring the entomological impact of larval control interventions against the mosquito vectors of dengue virus, they are not proxies for adult vector abundance. Neither are they useful for assessing transmission risk because they do not take into consideration the epidemiologically important variables, including adult vector and human abundance, temperature, and seroconversion rates in the human population.

The document reviews and critiques current methods, focusing especially on sampling methods that provide information on (1) the risk of transmission as a function of vector abundance, and (2) the relative or absolute importance of the various types of containers in the environment. This second aspect is essential when considering a suppression strategy designed to minimize costs or to improve sustainability by targeting only a subset of the breeding containers for control or elimination—specifically those container types that are responsible for the majority of adult production. In reviewing current and generally-used sampling methods, each is discussed with respect to transmission risk assessment and evaluated in terms of being useful for either “research or special studies” or as a practical operational tool providing useful information for planning and management of vector control programmes.

Introduction

This review was developed in response to a recommendation from the WHO Informal Consultation on Strengthening Implementation of the Global Strategy for Dengue Fever/ Dengue Haemorrhagic Fever Prevention and Control held in October of 1999 concerning “the refinement of existing indicators and/or the development of new **indicators that better reflect transmission potential.**” The Consultation recommended that “such indicators should provide clear, meaningful information for communities as well as for programme managers and policy-makers”.

Sampling methods and indicators of dengue vector abundance (primarily *Aedes aegypti* [L.]) are traditionally based on larval surveys of container habitats and the calculation of House, Breteau and Container indices. Such surveys and indices are of operational value and can facilitate the determination of local vector ecology and measurement of the impact of container-specific vector control interventions. However, they are a poor proxy for measuring adult abundance and are of limited use in assessing transmission risk.

This review critiques current methods but focuses especially on sampling methods that provide information on (1) the risk of transmission as a function of vector abundance and (2) the relative or absolute importance of the various types of containers in the environment. This second item is essential when considering a suppression strategy designed to minimize costs or improve sustainability by targeting only a subset of the breeding containers for control or elimination—those container types that are responsible for the majority of adult production.¹ In reviewing current and generally-used sampling methods, each will be discussed with respect to transmission risk assessment and evaluated in terms of being useful for either “research or special studies” or as a practical operational tool providing useful information for planning and management of vector control programmes.

Current sampling methods

Oviposition traps

Historically, ovitraps have provided useful data on the spatial (often in terms of simple presence or absence) and temporal (seasonal) distributions of *Ae. aegypti* and other container-inhabiting mosquitoes.^{2,3} Ovitrap data have also been successfully used to monitor the impact of various types of control measures involving source reduction and insecticides. Ovitrap data have been reported to be more sensitive than the traditional *Stegomyia* indices in detecting low populations.^{4,5} The use of a hay infusion instead of clean water results in significantly more attractive ovitraps which presumably translates into more sensitive surveillance.⁶ Chadee and Corbet used ovitraps to determine diel periodicity and oviposition behaviour of *Ae. aegypti* in Trinidad.⁷

If the goal is detection, i.e. the presence or absence of *Ae. aegypti*, surveys based on oviposition traps have been considered more cost-effective in addition to being more sensitive.⁸ The Singapore model of using 1700-1900 ovitraps to spatially target "hotspots" for additional source reduction efforts is an outstanding example of the utility and sensitivity of this approach (personal communication, Basil Loh). Mogi et al described the relationship between the mean number of eggs per ovitrap and the proportion of positive traps using a statistical model; using this relation, the mean number of eggs per trap, with confidence limits, can be estimated without egg counts.⁹

Theoretical problem with ovitrap data for assessing adult density

It is often not appreciated that ovitrap data are of questionable value when used to estimate differences in vector abundance between blocks or neighbourhoods. Consider a hypothetical example that highlights the principal confounding problem: two blocks, one with 10 outdoor drums positive for larvae, the other with 20, and each block with a single ovitrap. The average standing crop of *Ae. aegypti* adults would be expected to differ by a factor of roughly 1:2 and so would the average number of eggs laid each day. However, on the first block the oviposition goes back into 11 containers; on the second block with twice the number of adults and daily oviposition, the oviposition goes into roughly twice as many containers, viz. 21. The result is that the number of eggs *per ovitrap* is roughly the same. While seasonal variability in adult productivity can be monitored with the ovitraps, comparisons between areas at the same point in the season cannot be reliably made (see *Usefulness of ovitraps* below regarding their use in evaluating insecticide treatments).

Ovitraps and dengue transmission risk

Without a substantial time series of transmission and ovitrap data for a particular location, sufficient for the creation of a location-specific statistical relationship, one cannot assess risk of transmission with ovitrap data. Notice also that ovitrap data are even further removed than larval survey data are from providing information on which types of containers are most

important with regard to productivity. The reservation made in the preceding paragraph has application here as well. If a control programme reduced the number of wet containers, adult populations and total oviposition would be reduced; but this reduced oviposition would go into a reduced number of oviposition sites, and the statistic derived from ovitraps would remain independent of the actual reduction in adult levels. In contrast, a programme relying on larvicides or biologicals reduces the adult population without a corresponding reduction in containers; here ovitrap data would be expected to follow the trend in adult abundance. In reality, programmes often involve a mix of container elimination and control; in such situations different types of containers with differing productivities are influenced differently and meaningful interpretation of ovitrap data with respect to transmission risk assessment or population control may prove impossible.

Usefulness of ovitraps in control operations and/or research

It appears fully warranted to use ovitrap data to monitor the impact of insecticide treatments and the seasonality of adult populations.¹⁰ Their utility in locating areas for additional clean-up when *Ae. aegypti* populations are low is certainly justified, as the Singapore experience has shown. However, using ovitrap data as a proxy for reduced adult densities in programmes involving the destruction of containers or oviposition exclusion covers is of questionable value for the reasons outlined above. However, this author is unaware of any scientific studies that have attempted to relate ovitrap data (either percentage positive or average number of eggs per paddle) to adult densities with the exception of recent work in Cambodia where ovitrap, adult resting, and complete pupal survey data were collected simultaneously (personal communication, Nathan MB). Ovitrap data are inexpensive and it is possible to install them in large areas relatively quickly. They are amenable to use by people without specialized training for special studies and for evaluating certain insecticide-based operational programmes, e.g. space spraying.

Composite Index

Workers in Honduras developed a composite indicator to evaluate the impact of improved cleaning of water-storage containers by householders in a community-based *Ae. aegypti* control effort.¹¹ Containers subject to periodic cleaning had reduced adult production but traditional larval indices were not sensitive to this because the containers often had early instars present between cleanings. Their Composite Index was a summary measure of the degree of infestation of laundry basins (pilas) by *Ae. aegypti*. This index was the sum of four variables—presence of any immature stages (larvae and/or pupae), presence of pupae, detection of third-fourth-instar larvae in a five-dip net sample, and a log-transformation of the number of larvae recovered. The researchers found the new index to be more sensitive to changes in human behaviour resulting from a control programme exposure than a simple, dichotomous variable (i.e. positive/negative for presence of immature stages).

Usefulness of the Composite Index in dengue risk assessment, control operations and/or research

Several observations can be made in evaluating the utility of the Composite Index, not least that it is the most labour intensive of the methods discussed in this review. Since the goal was to monitor the impact of cleaning on adult production, it is not clear what was gained by adding the other three variables to pupal counts. There is no way to relate this index to transmission risk unless all containers were surveyed and a census was made of the number of people associated with them (as discussed below) so that the number of pupae per person could be calculated. For these reasons, the Composite Index has little utility in answering other research and control operations questions.

Adult collections

Resting boxes, landing collections, visual or sticky traps, and aspirator collections are important methods for the collection of male and female *Ae. aegypti* and it is interesting and informative to survey their varied applications.

Sticky traps. Bangs et al demonstrated that dengue-infected *Ae. aegypti* could be analysed for viral RNA using the reverse-transcriptase polymerase chain reaction assay (RT-PCR) for up to 30 days after capture on sticky papers attached to walls.¹² They believe the technique shows promise as a field tool for surveillance for the presence of virus in the community. Kay et al addressed the problem of sampling adult *Ae. aegypti* and other mosquitos which utilize subterranean habitats such as wells and service manholes using a sticky pipe trap.¹³ Their device was a small diameter cylinder, fitted with a screen on one end; depending on how the tube was oriented (up or down) when clipped to the undersides of service manholes, it was possible to identify the species, sex, and parity status by direction of movement. The tube was fitted with a strip of adhesive paper to retain the catch. As a final example, a sticky ovitrap was used to recapture marked *Ae. aegypti* in dispersal studies in Mexico.¹⁴

Aspirators and resting collections. The US Public Health Service developed a backpack battery-powered aspirator for the collection of resting adult mosquitos in and around human habitations.¹⁵ They noted that their aspirator facilitated the indoor collection of *Ae. aegypti* and provided information about the biology and behaviour of *Ae. aegypti* that was useful in education and vector control programmes and in the evaluation of ultra-low volume insecticide spray programmes directed against this species. A study in Puerto Rico investigating the association of 12 entomologic, environmental, and behavioural variables with the proportion of household members with laboratory-confirmed recent dengue infection, used the backpack aspirator for timed adult collections. They found that the only significant household risk factor for recent infection was the number of female *Ae. aegypti* per person.¹⁶ Edman et al studied the movement of marked *Ae. aegypti* as influenced by the relative availability of oviposition sites using backpack aspirators.¹⁷

Landing collections and visual traps. Using humans as bait for adult collections is expensive and poses safety issues in areas endemic for disease. Landing collections have been used

to study the diel periodicity of *Ae. aegypti* attraction to hosts.¹⁸ In studies in Cuba of the environmental and entomological determinants of dengue, it was concluded that resting collections were the most sensitive method of monitoring adult *Ae. aegypti* abundance.¹⁹

For some applications a visual trap may be useful and adequate. Several visual traps for *Ae. aegypti* and *Aedes albopictus* - the duplex cone trap,^a the Fay/Prince trap, the bi-directional Fay trap, and the omni-directional trap - were evaluated and compared to the standard US Centers for Disease Control and Prevention (CDC) miniature light trap.²⁰ They found that the bi-directional Fay and omni-directional traps collected significantly more *Ae. albopictus* females than did the other traps tested, and that the bi-directional Fay trap collected significantly more *Ae. aegypti* females than did any other trap. They concluded that these traps may be useful tools for sampling these species.

Usefulness of adult collections in control operations and/or research

Based on this sampling of adult collection methods, it is obvious that these techniques have been very useful and will continue to be so in a wide range of applications addressing a host of questions. Regarding sticky cards—when contemplating a system to detect virus in the field with sticky traps and RT-PCR, consideration should be given to the likelihood that the incidence of virus-positive female *Ae. aegypti* during the peak of acute epidemics will be very low, in the order of one per several thousand.²¹

Adult collections for dengue risk assessment

However useful these methods may be in a research or operational context, for two reasons they provide limited or no information to enable an assessment of transmission risk. First, the relationship between collections and absolute numbers of adults is unknown; adults rest indoors and out, often in inaccessible locations, and numbers collected only approach an estimate of total numbers of adults asymptotically with the amount of collection effort. The second hindrance in their use in risk assessment is that the relation between number of adults and transmission is unknown. Even with an associated count of humans, giving us adults per person, the relationship between risk and adults per person is not known. It would be possible to develop this relationship, as has been done with the easier-to-count statistic of pupae per person, using the dengue transmission models of Focks et al²¹ (described below), but the challenge of the first issue, estimating the absolute number of adults, still remains.

Passive larval and pupal collections

Kay and others have made a valuable contribution to sampling *Ae. aegypti* and other container-breeding organisms in sites with poor or difficult access, such as wells, with the development of the funnel trap.²² The device is basically a weighted funnel and bottle that inverts on entry to and exit from the water surface; the device collects organisms such as

^a. Note, unlike the other traps tested, the duplex cone trap uses CO₂ as an attractant.

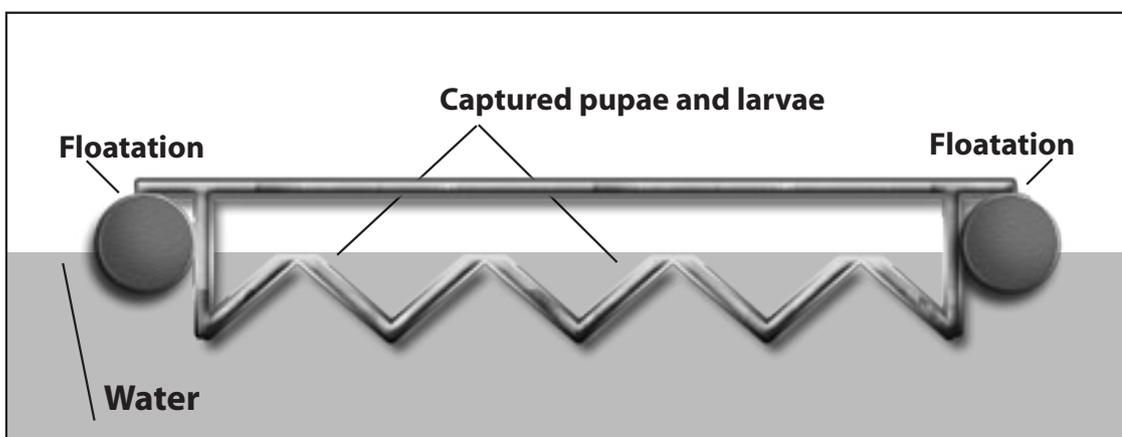
fish, copepods, mosquitos, ostracods, and tadpoles as they return to the surface. Calibration of the device using known numbers of *Ae. aegypti* larvae now enables estimation of larval population size.²³ The device has gained acceptance by surveyors and has focused attention on the fact that subterranean sites may serve as important sources of harbourage during winter or dry conditions.²⁴ The funnel trap captures a lower proportion of pupae because they are less active than larvae. To sample pupae, Focks (unpublished) developed a square floating device containing four parallel v-shaped troughs separated by narrow gaps at the top; the flotation is positioned such that the gaps are just submerged (see Figure 1 for a cross-sectional view) when the device is floating. The unit is repeatedly dropped into the water from a short distance, causing pupae to dive. The unit is then allowed to float on the water for a few hours during which time pupae returning to the surface come up under the device and are funnelled through the gaps and retained in the troughs. Using the ratio of the container's surface area to the area of the floating device, it may be possible to calibrate and make absolute population estimates.

Usefulness of passive collections in risk assessment, control operations and/or research

These passive collection methods should be valuable in control and research activities where important sources of *Ae. aegypti* include wells and other sites which are difficult to sample. Quantification of the funnel trap enables results to be compared with larval counts in other containers and allows estimates of the relative importance of the various types of containers to be made. However, there is no way to relate funnel trap captures to risk of transmission because there is no direct relationship between larval and pupal densities due to density-dependent larval survival.

Figure 1.

Floating frame with inverted louvres to retain surfacing pupae. The frame and louvres are made with light gauge sheet metal soldered together.



Traditional *Stegomyia* indices

The traditional *Stegomyia* indices - the House, Container, and Breteau - have been and continue to be the chief surveillance tool of many control programmes. However, these and related indices are increasingly being seen as inadequate to measure either the risk of transmission or the effectiveness of control operations; moreover, they provide no guidance regarding targeting control efforts.^{25, 26}

Historical background

During the initial efforts to control urban yellow fever in South America, it was observed that a substantial reduction in the number *Ae. aegypti* breeding sites would often eliminate transmission. This observation became the basis of the efforts organized in 1923 by the Rockefeller Foundation to eradicate yellow fever in coastal cities of northern Brazil.²⁷ Improved methods developed subsequently under Fred Soper resulted, quite unexpectedly, in the eradication of *Ae. aegypti* in several cities in 1933. The goal of *vector* eradication arose later in Brazil, not as a requirement for yellow fever eradication, but rather, from a desire to protect *Ae. aegypti*-free zones from re-infestation.²⁸

To monitor vector control progress and to determine if prophylactic levels have been achieved, *Stegomyia* indices were developed.^{29, 30} The initial indices, described in 1923, were the House (or Premises) Index (HI)—the percentage of houses infested with larvae and/or pupae, and the Container Index (CI)—the percentage of water-holding containers infested with active immatures; 30 years later, the Breteau Index (BI)—the number of positive containers per 100 houses, became a common measure.³⁰ In the late 1960s, the World Health Organization began promoting the world-wide surveillance of *Ae. aegypti* and related species;³¹ to facilitate the dissemination of this information on maps, a statistic was developed - the *Density Figure or Index* (DI), and then empirical relationships between it and the *Stegomyia* indices were derived (Table 1).³² More recently, Tun-Lin et al developed

Table 1.

Aedes aegypti Density Figure and corresponding *Stegomyia* indices after the work of AWA Brown.^{32,33}

Density Figure	House Index	Container Index	Breteau Index
1	1-3	1-2	1-4
2	4-7	3-5	5-9
3	8-17	6-9	10-19
4	18-28	10-14	20-34
5	29-37	15-20	35-49
6	38-49	21-27	50-74
7	50-59	28-31	75-99
8	60-76	32-40	100-199
9	>77	>41	>200

and evaluated a new measure, the Adult Productivity Index (API); it is based on the sum of container type frequency multiplied by a density figure representative of each type.²⁶ The motivation for developing this new measure was to take into account the differences in container abundance. However, in an extensive evaluation of the API, the authors found it to be no better than the Breteau Index.²⁶

Epidemiological significance of the Stegomyia indices

A number of workers have suggested that these indices have epidemiological significance. For example, Connor and Monroe, developers of the HI and CI, observed in 1922 that a CI $\leq 10\%$ in urban areas of Central and northern South America constituted a “safety zone” regarding yellow fever transmission;²⁹ Soper gave a prophylactic level for the same tropical areas to be a HI of $< 5\%$.²⁷ AWA Brown, the developer of the DI, noted that transmission in the 1965 yellow fever epidemic in Diourbel, Senegal, occurred where CIs were > 30 and BIs were > 50 (DIs > 5) and not where the BIs were < 5 (DI = 1).³³ Brown also mentioned, with respect to dengue in Singapore, that dengue haemorrhagic fever (DHF) was most prevalent where HIs were > 15 , corresponding to DIs > 3 . It is important to note that critical thresholds in terms of these indices have never been developed for the dengue system.³⁴

Shortcomings of the traditional Stegomyia indices

Variations on this idea of positive containers per person or area have been developed, but the essential notion is the same and the following shortcomings of the traditional measures hold for them as well.

It is important to note that the ability to infer a relationship between one or more of these indices and transmission was developed^a empirically over many years and only for a particular region. Moreover, there is no mechanism for using these indices to adjust risk assessments or estimates of required suppression levels for the significant influences of herd immunity or anomalous temperatures associated with, say, El Niño/Southern Oscillation events. Nor do they provide reliable information regarding the epidemiological significance of the various classes or types of containers, e.g. flower vases, drums or tyres, which differ substantially in their daily production of adult *Ae. aegypti*. In light of these shortcomings (further detailed below), it is not surprising that, while programmes dutifully go about estimating the various traditional indices, these are virtually never used for risk assessment and provide only minimal guidance to control programmes.

It has recently been argued that the *Stegomyia* indices as epidemiologic indicators of dengue transmission should be viewed with caution.^{25,26} The traditional indices have a number of serious shortcomings. The CI is probably the poorest since it reflects only the proportion of containers positive in an area and does not take into account the number of containers per area, per house, or per person. The HI is perhaps better, but this index does not give the number of positive containers per positive house. Of the indices, the BI has the advantage of combining information on containers and houses.²⁶ However, all three indices fail to take into account that containers vary in the production of adult *Ae. aegypti*. For

example, an indoor flower vase may commonly be found with larvae but seldom produces an adult because of frequent water changes, whereas, say, an uncovered, outdoor 220 litre drum under a tree may support a standing crop of 10, 20 or even 50 pupae. Yet, for the purposes of calculating the indices, they are equally positive. Field observations bear this out: Southwood et al reported, for a temple area in Bangkok, an approximately 23-fold difference between the most and least productive types of container.³⁵ A six-fold difference was seen in Honduras.²¹ Connor and Monroe, in their original paper on indices, recognized these shortcomings and, in 1923, pointed out that herd immunity was an additional and important epidemiologic factor not considered by the *Stegomyia* indices.²⁹ An additional shortcoming is that these indices fail to adequately provide data on a per area or per person basis, factors which are known to relate to levels of transmission.^{21,36,37,38,39,40}

Insofar as each of the *Stegomyia* indices is considered to be a proxy for the same thing, namely transmission risk, they should be positively correlated; the very existence of Table 1 implies such relationships. In regard to this assumption, the results of a recent container survey of 100 houses in each of 16 towns in Trinidad that permitted the simultaneous estimation of the indices are enlightening.²⁵ In this study, correlations between the BI, HI, and CI (Table 2) were significant from a statistical point of view, i.e. all *P-values* were ≤ 0.05 ; however, the actual amount of variation (i.e. R^2 or coefficient of determination) in one index that could be explained by reference to another index ranged between 30% (CI vs. HI) and

Table 2.

Pearson product moment correlations and associated *P-values* (in italics) between traditional *Stegomyia* indices and pupal measures in the Trinidad dataset.²⁵ Significant correlations ($P \leq 0.05$) are indicated with an asterisk.

Measure	Pupae per hectare	Pupae per person	Breteau Index	Container Index	House Index
Pupae per hectare	1.000	0.963	-0.119	-0.522	0.121
	<i>1.000</i>	<i>0.000*</i>	<i>0.660</i>	<i>0.038*</i>	<i>0.660</i>
Pupae per person	0.963	1.000	-0.181	-0.535	0.117
	<i>0.000*</i>	<i>1.000</i>	<i>0.502</i>	<i>0.033*</i>	<i>0.665</i>
Breteau Index	-0.119	-0.181	1.000	0.590	0.683
	<i>0.660</i>	<i>0.502</i>	<i>1.000</i>	<i>0.016*</i>	<i>0.004*</i>
Container Index	-0.522	-0.535	0.590	1.000	0.552
	<i>0.038*</i>	<i>0.033*</i>	<i>0.016*</i>	<i>1.000</i>	<i>0.027*</i>
House Index	0.121	0.117	0.683	0.552	1.000
	<i>0.660</i>	<i>0.665</i>	<i>0.004*</i>	<i>0.027*</i>	<i>1.000</i>

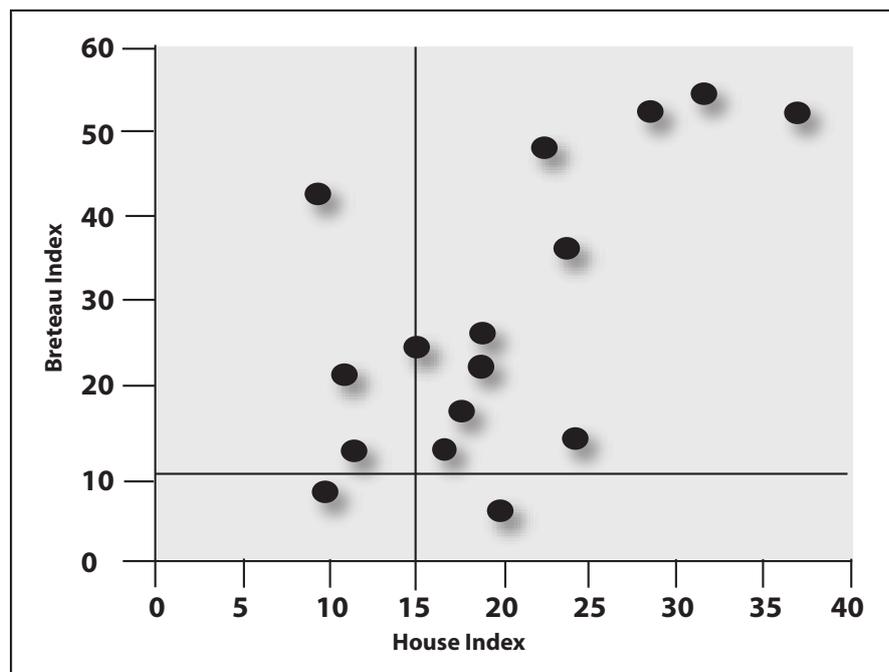
47% (BI vs. HI). The relationship between the BI and HI shown in Figure 2 is typical of all three correlations and it gives an indication of the poor relationship between the traditional indices. Table 1 was developed by AWA Brown using data from 172 surveys where all three indices were simultaneously calculated. The BIs were grouped into nine classes and the *average* HI and CI values were plotted against the *average* BI for each group. However, no documentation on the *variability* of these relationships was provided.³²

Usefulness of the traditional Stegomyia indices in dengue risk assessment and control operations and/or research

In the section below on the pupal and demographic survey, the issue of correlation between the indices and the absolute abundance of *Ae. aegypti* is again considered and the case made that the indices provide essentially *no* information on transmission risk or the identification of important classes of containers for targeted source reduction/control operations. On empirical grounds, scepticism is understandable in light of numerous occasions where

Figure 2.

Plot of Breteau Index versus House Index for the Trinidad study; correlations and *P-values* are presented in Table 2. If we use AWA Brown’s observations on DHF being found in Singapore where HIs > 15, corresponding to a BI of ca. 11 (Table 1), we see significant inconsistencies. According to the HI, four locations are safe that the BI identifies as unsafe. Alternatively, the BI mis-identifies a single town suggested by the HI statistic to be safe. Notice as well the string of four BI points ranging between 10 and 42, all associated with an invariant HI value of ca. 11.



outbreaks occurred after control efforts resulted in *safe* indices.^{34,41, 42, 43}

Increasingly, operational programmes are realizing that the control of some types of containers is more important than of others; this is not usually based on pupal surveys (described below) but on general observations reflecting the combination of container abundance and some unquantified notion of productivity. An obvious and necessary means of monitoring progress in such a targeted approach would, for containers that can be removed, be to compare their pre-treatment and post-treatment numbers in the environment using, as a denominator, the number of houses surveyed, e.g. the average number of tyres per house.

The development of transmission thresholds and use of the pupal/demographic survey

Today, most dengue control efforts are based on suppression of *Ae. aegypti* and not on eradication.^{44, 45} Such efforts would benefit from answers to the following questions: (1) How much suppression is adequate to be prophylactic for a particular location? (2) How do we monitor the degree of suppression achieved in ongoing programmes? Some have recently been advocating an additional question: (3) Given that the epidemiological importance of a particular type of container is the product of the average adult productivity and the abundance of that type of container in the environment, how do we select subsets of container types for intervention such that we optimize labour efficiency and cost while maximizing adult reductions?^{1,36}

Answering the first question involves a discussion of transmission thresholds—what they represent and how they have been estimated. As outlined below, thresholds are a function of many factors, but a key factor is the statistic ‘adult *Ae. aegypti* per person’, hence the need for a survey method that permits estimation of this important variable. Because of the difficulty of estimating absolute adult density, and given that pupae can be counted, plus the fact that the latter are highly correlated with the standing crop of adults, a survey method involving counting pupae and people has been developed.²⁵

The pupal/demographic survey method

In practice, conducting a pupal and demographic survey involves the visiting of 100^b or more residences, usually by a pair of inspectors equipped with nothing more than a few litres of clean water, a sieve,^c some large-mouth pipettes, a white enamel pan, and small shell vials. The inspectors request permission to examine the water-holding containers and enquire as to the number of people living at the house (or sleeping there the preceding night). With permission, they proceed to strain each container at the location, re-suspending the sieved contents in a small amount of clean water in the enamel pan. From there the pupae are pipetted into a labelled vial. Methods to estimate the number of pupae from sweep nets in lieu of a total pupal count for large containers are presented below. If there are other container-inhabiting species in the area besides *Ae. aegypti*, the contents of each vial are

^b As will be seen in the section on the contagious and non-normal distribution of pupae per container, the number of premises needed for an adequate survey, i.e. sample size, is an important research area.

^c USA Standard Sieve Series Number 30 sieve (equivalent to ASTM designation E11, 600 µm (0.0243”) opening).

transferred to small cups covered with bridal veil secured with a rubber band; these are held in the laboratory or other suitable room until adult emergence occurs and taxonomic identification can be made. A key for container-inhabiting mosquito pupae from South-East Asia has recently been published.⁴⁶ Data are usually summarized by container type in a spreadsheet.

The centrality of transmission thresholds in epidemiologic risk assessment

Theoretical underpinnings

Estimates of transmission thresholds for dengue based on the number of *Ae. aegypti* pupae per person were based on a pair of simulation models (CIMSiM/DENSiM) developed to provide site- and weather-specific insight into the dynamics and control of dengue viruses and their vectors.^{21,47,48} These models reflect a long history of mathematical modelling of epidemiologic phenomena. As early as 1906, Hamer postulated that the course of an epidemic depended on the rate of contact between susceptible and infectious individuals,³⁷ this notion, *the mass action principal*, has become a central concept in mathematical epidemiology—the rate of spread of an infection within a population is proportional to the product of the density of susceptible and infectious people. Ross used this principal in his pioneering work on the dynamics of malaria transmission.³⁸ The insight of Hamer and Ross was further developed by Kermack and McKendrick in 1927 into an understanding of the concept of thresholds.³⁹ Anderson and May consider this *threshold theory*, coupled with the mass action principal, to be the cornerstone upon which modern epidemiological theory is built.⁴⁰ The notion of thresholds indicates that the introduction of a few infectious individuals into a community of susceptibles will not give rise to an epidemic outbreak unless the density of susceptibles (or vectors) is above a certain critical level.

Why count pupae and people?

It is obvious that applying threshold theory in the context of dengue risk assessment and control will involve knowing, in absolute terms, the ratio of *Ae. aegypti* females to humans. For the reasons cited above in the section on adult collection methods, it is simply not possible to accurately and inexpensively measure adult abundance in the field; a proxy is needed, something that is highly correlated with adult density and expressed as a ratio per person. Pupae are used for several reasons: 1) unlike any of the other life stages, it is possible to actually count the absolute number of *Ae. aegypti* pupae in most domestic environments;^{35,25} 2) container-inhabiting *Stegomyia* pupae are easily and inexpensively separated from other genera and identified to species as emerged adults or pupae;⁴⁶ 3) because pupal mortality is low and well-characterized, the number of pupae is highly correlated with the number of adults.⁴⁷ Importantly, counting pupae also permits evaluation of the relative and absolute contributions of the various classes of containers. On the other hand, counts of eggs or larvae are considered unsuitable proxies for two reasons: the labour needed for such

counts is prohibitive, and delayed hatching and variable mortality in the egg stage and density-dependent larval mortality make any determination of their relationships to adult populations virtually impossible.

Transmission thresholds

The development of thresholds for dengue has been presented in some detail above.³⁶ The definition of an epidemic is arbitrary but useful from a public health point of view—any single year where seroprevalence rises by at least 10% is considered to be an epidemic year. Ten per cent was selected because any disease involving that proportion of the population would be considered an epidemic and this level of transmission would result in just slightly more than 1% of the population being infected during the peak of the epidemic—a minimum value that has been suggested as sufficient for the detection of transmission.⁴⁹ Just how many mosquitos per person are required to support this level of transmission is a function of many factors, but the ones considered key determinants are the seroprevalence of dengue antibody and temperature. In these assessments several important assumptions have been made that are likely to be true in most tropical locations: 1) vector competence is adequate; 2) blood feeding by *Ae. aegypti* occurs primarily (> 90%) on humans; 3) essentially all hosts are at risk of being bitten. Conditions 1 and 2 are unlikely to be true in the south-eastern United States and constitute an obvious exception to these assumptions. Table 3 contains transmission thresholds for dengue in terms of *Ae. aegypti* pupae per person as a function of temperature and herd immunity.³⁶

Table 3.

Estimated number of *Ae. 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 or two viraemic individual(s) on day 90 of the year; the estimates for two individuals are in parentheses. In a series of simulations in DENSIM, these values resulted in a 10% or greater rise in prevalence approximately 50% of the time.³⁶

Temperature (°C)	Transmission thresholds by initial seroprevalence of antibody		
	0%	33%	67%
22	9.57 (9.16)	14.10 (12.83)	30.55 (29.15)
24	2.92 (2.68)	4.47 (4.21)	9.22 (8.68)
26	1.42 (1.23)	2.03 (1.98)	4.26 (4.01)
28	0.53 (0.48)	0.75 (0.72)	1.69 (1.38)
30	0.13 (0.12)	0.19 (0.18)	0.38 (0.35)
32	0.07 (0.07)	0.10 (0.10)	0.26 (0.18)

The use of transmission thresholds and the pupal/demographic survey in source reduction programmes

The underlying notion of targeted source reduction is one of selectively attacking the most important types of containers. As presented earlier, field observations suggest the rationale is sound in that containers vary significantly in their production of *Ae. aegypti*. The actual epidemiologic significance of any particular type of container, say discarded tyres, is a function of the average standing crop of pupae found in that type and the abundance of that container.²⁵ Table 4 is an example of how transmission thresholds and the pupal and demographic survey could provide guidance to a targeted source reduction effort. The estimate of the transmission threshold provides an overall target, an upper limit on the number of pupae per person for the environment that ensures that viral introductions would result in little or no transmission. The survey permits estimating the contribution of each type of container and allows, using nothing more than a spreadsheet, conducting what-if analyses of various strategies designed to selectively attack different types of containers at various rates of elimination based on their epidemiologic importance and how amenable they are to elimination and/or control.

The following example is based on surveys conducted during June 1995 in urban areas of central St. George County in northern Trinidad.²⁵ Based on average temperatures for this period (27.8°C) and assuming a seroprevalence rate of 33%, the estimate of the transmission threshold is approximately 0.71 pupae per person (interpolation of Table 3). The surveys estimated human densities to be ca. 160 per hectare and provided data on the nine major types of breeding containers, their abundance, and average standing crop of *Ae. aegypti* pupae (Table 4). In this environment, there was an average of approximately 98 water-filled containers and 209 pupae per hectare; the number of pupae per person was 1.31 or 184% of the threshold. Numerically, the two most common types were indoor containers: the flower vase and water storage drum. It should be noted, however, that they differed significantly in productivity. The epidemiologic significance of the indoor water storage drum, based on its contribution to the number of pupae per hectare or per person, was some 40-times greater than the flower vase. Dividing the estimate of pupae per person for each container type by the threshold of 0.71 yields an estimate of the proportion of the threshold which is contributed by it; this indicates that the vases contributed < 2% of the threshold whereas the indoor drums accounted for > 70%. Again, from Table 4, targeting of the more important container types based on this logic would suggest a focus on indoor and outdoor water storage drums and perhaps the tubs. If Table 4 is put into a spreadsheet, evaluating various targeted strategies becomes easy. It can be seen that an overall reduction of about 50% of all containers would result in the number of pupae per person being reduced to approximately 92% of the threshold and would require the control or elimination of about 50 of the 100 containers per hectare. However, with a targeted approach that controlled or eliminated about 55% of the three most important types, i.e. the indoor and outdoor drums and the tubs, the number of pupae per person would be reduced to approximately 93% of threshold, and would require the control of only about 23 containers per hectare. This approach would help programme managers to decide on which container types to concentrate their efforts, and to what degree, bearing in mind that some types are more or less controllable by virtue of their location, ownership, use, etc.

Practical aspects of using thresholds and the pupal/demographic survey

For a control operation, it is recommended that an initial and careful survey of the standing crop of pupae in all containers associated with 100 or more houses be made. Provided the areas to be controlled are similar to the survey area, risk assessments and control strategies for other areas use only counts of containers by type and the numbers of humans; the estimates of the average standing crop of *Ae. aegypti* pupae per container from the detailed surveys are used for the calculations for the additional areas. And in monitoring the progress of operations, only counts of uncontrolled containers are used and a full pupal/demographic survey is not conducted.

Important observations on various distributions that impact control programmes and the size of surveys

In an effort to evaluate the utility of predaceous *Toxorhynchites* for control of peri-domestic *Ae. aegypti*, a simulation analysis led to the conclusion that the key factor regulating the degree of possible control was the overlap in the ovipositional preferences of the two species.⁵⁰ This result led to a census of all active immatures in a six-block area of New Orleans, Louisiana, USA; the goal was to determine what proportion of the production of *Ae. aegypti* came from containers that were suitable to *Toxorhynchites*

Table 4.

An example of survey results from urban sites in St. George's County, Trinidad, conducted during June 1995 and incorporating a transmission threshold estimate of 0.71 pupae per person. The threshold estimate is based on interpolating values in Table 3 using an average June temperature of 27.7°C and an overall seroprevalence of 33%. *Pupae per ha* is the product of *containers per ha* and *pupae per container*. *Pupae per person* is the ratio of *pupae per hectare* and the average, observed human density of 160 per ha. *Portion of threshold* is the ratio of *pupae per person* and the threshold estimate. *Relative importance* is the ratio of *pupae per person* for each container type and the total number of pupae per person, 1.307. Putting data like these and their relationships into a spreadsheet permits 'what-if' analyses of the anticipated impact of various targeted source reduction strategies.

Container type	Containers per ha	Pupae per container	Pupae per ha	Pupae per person	Portion of threshold	Relative importance
Saucer	3.9	0.20	0.8	0.005	0.007	0.004
Tyre	0.8	1.00	0.8	0.005	0.007	0.004
Small misc.	1.2	1.10	1.3	0.008	0.012	0.006
Indoor vase	40.0	0.05	2.0	0.013	0.018	0.010
Tank	9.5	0.40	3.8	0.024	0.034	0.018
Bucket	1.1	10.90	12.0	0.075	0.106	0.057
Tub	13.5	3.80	51.3	0.321	0.452	0.245
Outdoor drum	8.3	6.70	55.6	0.348	0.490	0.266

females for oviposition.⁵¹ The results have very important ramifications for targeted source elimination and/or control, where the goal is control or elimination of only a sub-set of all container types. The proportion of blocks positive for *Ae. aegypti* ranged between 0.14 and 0.55 (a 3.9-fold difference). The number of positive containers per block ranged from 5 to 43 (8.6-fold difference) and the number of pupae per block, the study proxy for productivity, ranged from 49 to 875 (a 17.9-fold difference). The number of foci per house was also decidedly non-normally distributed, with some 56% having no breeding foci and 2% having 7-11. The distribution of the numbers of wet containers (and foci) by size was again not normal but highly skewed with container size being inversely related to abundance. And finally, the number of pupae per container was highly skewed with most having none and the odd container having 30 or 70 or 280.

If we assume that we cannot identify, in a cost-effective (and sufficiently accurate) fashion, those particularly productive blocks or houses or containers, we are forced to a strategy outlined above where we target the entire population of containers—independent of productivity—going to every house on every block. However, if sufficiently reliable surrogates for productivity, at the scale of blocks, houses, and/or containers, can be developed, the potential exists to significantly reduce costs through a targeted approach. In New Orleans, unmown lawns and peeling paint on houses was a reliable indicator of *Ae. aegypti* production at the block and house level (here we have a potential surrogate, one of *condition*). Low (or no) production was common in containers in the immediate vicinity of the house; big producers were unused and abandoned containers commonly found at the back of the property (a surrogate involving *location* and/or *use*). Tyres and bottles, accounting for 2.5 and 26% of all wet containers, differed in their average standing crop of pupae such that tyres accounted for 26% of all production with bottles being associated with < 0.1% (a productivity surrogate of *container type*). Perhaps the current vision of differences in productivity, e.g. *key containers*, should be expanded to include surrogates other than simply container type.⁵²

Productivity and correlations between indices and abundance

Sweep nets and estimating container productivity

In this context, some recent work from Australia by Tun-Lin et al is important and encouraging. Tun-Lin begins by noting that the traditional *Stegomyia* indices do not correlate with adult female abundance and dengue risk, and proposes to improve sampling and risk assessment methods by taking productivity into account.^{53,42,43} Because their proxy for productivity is primarily larvae, they experimentally established correlations between the standing crop of larvae (and pupae) with sweep net captures in drums; the results are promising with correlations ranging between 0.92 and 0.96.⁵³ This work is important because it permits the estimation of productivity in large containers that are otherwise difficult and expensive to count.

Other researchers are recognizing the need to take productivity into account when directing control efforts. For example, a recent dengue control effort in Colombia used

sweep nets to estimate productivity of the various types of containers; this information was then used to direct targeted interventions.⁵⁴ Martinique has developed a modified BI that reflects, in a semi-quantitative manner, the differences in average production by container type; this approach permits adaptation of the health education messages by geographical sector on the island.⁵⁵ A final example was the targeted application of temephos into water storage jars in an emergency anti-dengue campaign in Cambodia that significantly reduced transmission (personal communication, Nathan MB, Olson JG).

Streamlining surveys by focusing on particular premises - the Premise Condition Index

In an effort to facilitate the location of positive premises and containers in Queensland, Tun-Lin et al used various forms of statistical analyses to develop the Premise Condition Index (PCI)—in essence, they were looking for proxies or surrogates to detect the presence of high level outliers among containers and premises.⁵⁶ They found that the condition of the house, the degree of shade and tidiness of the yard, both observable without entering the house or yard, were strongly correlated with both the proportion of positive premises and the numbers of infested containers, and they concluded that the PCI is a useful assessment tool that can increase the efficiency of detecting positive premises and containers for subsequent survey or control efforts. If only premises with PCI scores of 8-9 were surveyed, they found that the probability of finding a positive premise and positive container was increased 270% and 370%, respectively; these houses represented 9.5% of all premises yet accounted for 35% of all positive containers. An important observation was that positive houses were more than three times more likely to remain positive over time than a negative premise was to become positive over the course of a year.^{57, 58} If control resources are limited, it makes sense to focus on former key premises rather than use a random or systematic approach.

Premise and container productivity - distributions and stability

Tun-Lin et al analysed two sequential surveys, conducted in 1989 and 1990, of three locations in Queensland involving more than 1300 premises.⁵⁸ They found that a small proportion of premises was responsible for the majority of production; these foci of breeding were called *key premises*. As an example, < 2% of inspected premises in Townsville accounted for 47% of all positive containers in 1989; in 1990, 3% of all premises accounted for 53%. They also determined that some container types produced more than others and called these types *key containers*. Examples of especially productive containers included wells and rainwater tanks. They noted that averaging the productivity of each type of container provides a spatially and temporally stable estimate of the productivity of that class and that this average could be used with the abundance of the class to estimate relative importance. In summary, the concepts of *key premise* and *key container* reflect the decidedly non-normal distribution of productivity.

Observations on the clumped distribution of pupae per container

This section presents some unpublished results from pupal/demographic surveys conducted in the Americas and S. E. Asia suggesting that it may be possible to develop a dengue control strategy which focuses on rare but particularly productive containers and ignores the others.⁵⁹

The distribution of pupae per container is highly clumped

While in the process of spatially modelling *Ae. aegypti* pupae per person in an ongoing study in Iquitos, Peru, Dr. Subhash Lele of the University of Alberta and this author noted that the number of pupae per container was not normally distributed—rather, it was clumped with surprisingly few containers accounting for essentially all adult production; this was consistent with similar observations in New Orleans on the distributions of *Ae. aegypti* pupae per container.⁵¹ Subsequent evaluation of pupal-demographic survey data from Peru, Mexico, Puerto Rico, USA, and Indonesia revealed very similar clumping. This led to an assessment of the potential utility of targeting this very uncommon, but very productive, portion of the water-holding containers in the environment for dengue control. The following conclusions were reached: 1) the number of *Ae. aegypti* pupae per container was not normally distributed but highly clumped (i.e. aggregated, contagious), with most containers having none, a few having 1-10, and a very few (perhaps < 1% of all containers) having > 98% of the total of pupae; 2) these highly productive, but very rare, containers contributed the bulk of all adult production in the environment - Tun-Lin and Kay made similar observations in Australia and Fiji;^{58,52} 3) in some locations, these productive containers had characteristics that would facilitate their location and hence control; and 4) a strategy of targeted source reduction directed solely against this type of highly productive container, while ignoring the bulk of the water-holding containers in the environment, would suffice to control dengue and has the possibility of being sustainable.

Some examples from the Americas and S. E. Asia posing the question of targeting relatively few containers for control or elimination

Some classes of containers, by virtue of the product of their abundance and average standing crop of *Ae. aegypti* pupae per container, are undoubtedly more important epidemiologically than others. And standard epidemiologic theory and observation supports the notion of transmission thresholds and prophylactic targets of maximum numbers of pupae per person. Moreover, the tables of field survey data below indicate that especially productive containers account for the majority of adult production.

Nevertheless, there are many practical questions that need to be addressed before appropriate intervention strategies and behavioural messages for communities are developed for targeting certain containers. The surveys reported below simply used physically descriptive container names, sometimes with an indication of whether the

container was indoors or outside. However, it can be appreciated that such categorization is insufficient for describing certain important characteristics of containers. For operational reasons there is utility in classifying containers from a number of different perspectives, taking into consideration observations with regard to location (indoors, immediately outdoors, outdoors in areas of the yard that are overgrown, etc.), frequency of use (daily use, occasional use, abandoned), ownership (belonging to residents, abandoned on public property/rights of way), actual nature of the container (vase, tyre, drum, etc.), and functionality (for storage of drinking water or for wash water, etc.). Because of the potential utility of controlling only a subset of the containers which are more important than others, meaningful classification schemes become essential. For example, functional classifications, e.g. water storage jars for daily use or long-term storage, may warrant development of different socially or culturally appropriate management strategies. The tables below may provide sufficient encouragement to evaluate these concepts systematically for application in other locations.

The results reported here are of surveys conducted in Myaguez and San Juan, Puerto Rico, Iquitos, Peru, Reynosa, Mexico, and Yogyakarta, Indonesia. Table 5 provides information on the size of each survey in terms of people, area, and number of containers. The number of houses surveyed ranged from approximately 50 to 600; human density ranged from approximately 100 to > 600 per hectare. The number of artificial containers per person varied more than ten-fold—0.8 to 8.6 per person. The physical area surveyed ranged between approximately 1 and 11 hectares. As yet, the required survey size, in terms of area or premises, has not been determined, but it is acknowledged to be some function of how rare and productive are the rare and productive containers.

Table 6 provides survey results from these five locations and estimates of the transmission threshold based on the average temperature of the hottest month of the year and an assumed seroprevalence of 33%. The variable *proportion of threshold* is the ratio of the observed number of *Ae. aegypti* pupae per person to the estimated *transmission threshold*. If the ratio is greater than one, viral introduction would be likely to result in substantial

Table 5.

Information on pupal-demographic surveys - area surveyed and numbers of artificial containers (ACs), and total numbers of houses and people.

Site	Area (ha)	Numbers of		Per hectare		AC/person
		Houses	AC	People	AC	
Myaguez, Puerto Rico	0.8	46	123	149	160	0.8
San Juan, Puerto Rico	1.3	45	193	105	152	1.8
Iquitos, Peru (Mynas)	10.5	592	2569	298	246	8.6
Reynosa, Mexico	1.8	46	149	117	81	1.3
Yogyakarta, Java	4.6	324	3357	626	733	5.4

transmission. Under the heading *reduction required*, two variables are considered, *not targeted* and *targeted*: under *not targeted* are estimates, for each location, of the proportion of the total number of containers that needs to be controlled/eliminated for the neighbourhoods to be near their thresholds, assuming there is no targeting of especially important containers and that the number of pupae per container is normally distributed. Under *targeted* are the proportions of containers that need to be controlled/eliminated if the strategy focuses only on the most productive containers in the environment. Note that controlling the important containers reduces the overall number of containers which the community must tackle. However, development of such a strategy is contingent on developing an appropriate classification scheme and interventions which facilitate their location and control. Tables 7-9 provide details of three separate surveys and list specific containers, their associated number of *Ae. aegypti* pupae, and what proportion of the transmission threshold each container accounted for. The lists have been sorted from highest to lowest number of pupae per container; containers without pupae are not listed.

Table 6.

Survey data from five localities - *temp* is the average temperature of the hottest month of the year; *transmission threshold* is based on that temperature and an assumed seroprevalence of 33%. *Proportion of threshold* is the ratio of the observed number of *Ae. aegypti* pupae per person to the estimated *transmission threshold*. *Reduction required*, under *not targeted*, is an estimate of the proportion of the total number of containers that needs to be controlled or eliminated for the neighbourhoods to be near their thresholds, assuming no targeting of especially important containers. *Targeted* is the proportion of containers that needs to be controlled or eliminated if the intervention focuses only on the most productive containers in the environment. Note that controlling the important containers reduces the overall number of containers the community must deal with. However, development of such a strategy is contingent on developing an appropriate classification scheme and interventions which facilitate their location and control.

Site	Temp (C)	Ae. aegypti pupae		Transmission threshold	Proportion of threshold	Reduction required (%)	
		Total	Per person			Not targeted	Targeted
Mayaguez, Puerto Rico	26.9	515	4.48	0.90	5.0	80.0	2.4
San Juan, Puerto Rico	29.2	395	2.97	0.29	10.2	90.0	9.3
Iquitos, Puerto Rico	27.1	2,821	0.91	0.82	1.1	10.0	0.1
Reynosa, Mexico	30.8	608	2.84	0.13	21.2	95.0	20.8
Yogyakarta, Java	27.5	2,136	0.74	0.67	1.1	10.0	0.3

Yogyakarta, Indonesia (Table 7). Of 3357 containers associated with 2869 people, only 202 had one or more pupae; listed are the ones with > 15 *Ae. aegypti* pupae. *O* and *I* refer to outdoor and indoor locations. Controlling the nine most productive containers would reduce the community to below the transmission threshold. However, controlling two types of containers within the bathroom environment, the *bak mandi* (container holding water to flush the toilet) and *bak air* (container holding water used for bathing), and ignoring all other containers types, would reduce the number of pupae per person to below the threshold. An important point here is that only a very few containers out of thousands accounted for essentially all production.

Mynas neighbourhood in Iquitos, Peru (Table 8). In this setting, the most productive containers were unused, infrequently used, or abandoned large, rain-filled containers, mostly in the backs of the backyards. Only a few (mostly large, abandoned drums and similar containers located on the back property line) would need to be controlled to be prophylactic. No attention would need to be given to any of the indoor containers or backyard containers associated with water storage or clothes washing. This appears to be a clear example in which the targeting of a single class of container would be sufficient for prophylaxis. Under such circumstances, formative research and intervention development might be focused solely on these containers. Alternatively, inspectors with a search strategy only for these containers could inspect hundreds of houses per day, while ignoring the rest, and treating or eliminating only those found with large numbers of pupae.

Rio Cristal, Mayaguez, Puerto Rico (Table 9). Forty-six houses were surveyed; 123 water-filled containers were counted and only 16 had one or more *Ae. aegypti* pupae. The top two producers (a sink and a drum) were unused containers at one house. All major producers were situated outdoors. Elimination of three of the 16 positive containers would be prophylactic. This table focuses attention on the key unknown—is it possible to develop an adequate classification scheme that would permit targeting?

Table 7.

Yogyakarta, Indonesia. Of 3357 containers associated with 2869 people, only 202 had one or more pupae; listed are the ones with >15 *Ae. aegypti* pupae. O and I refer to outdoor and indoor locations. Controlling the nine most productive containers (shaded) would reduce the community to below the transmission threshold. However, controlling two *types* of containers within the bathroom environment, the *bak mandi* and *bak air*, and ignoring all other container types would reduce the number of pupae per person to below the threshold. An important point here is that only a very few containers out of thousands accounted for essentially all production.

Description	Number of pupae	Proportion of total	Pupae/person	Proportion of threshold	Cumulative pupae/person
O-Bak sampah	191	0.089	0.067	0.099	1.111
I-Bak mandi	162	0.076	0.056	0.084	1.012
I-Bak mandi	123	0.058	0.043	0.064	0.928
I-Bak air	96	0.045	0.033	0.050	0.864
O-Kolam ikan bekas	86	0.040	0.030	0.045	0.814
I-Bak mandi	66	0.031	0.023	0.034	0.769
O-Ember	54	0.025	0.019	0.028	0.735
I-Bak mandi	48	0.022	0.017	0.025	0.706
I-Bak mandi	47	0.022	0.016	0.024	0.682
O-Bak air	47	0.022	0.016	0.024	0.657
I-Bak air	44	0.021	0.015	0.023	0.633
O-Bak air	37	0.017	0.013	0.019	0.610
I-Bak mandi	35	0.016	0.012	0.018	0.590
O-Bak air	34	0.016	0.012	0.018	0.572
O-Kaleng bekas	34	0.016	0.012	0.018	0.555
O-Ember	30	0.014	0.010	0.016	0.537
O-Kaleng bekas	30	0.014	0.010	0.016	0.521
O-Pot bunga	30	0.014	0.010	0.016	0.506
I-Bak mandi	27	0.013	0.009	0.014	0.490
O-Bak mandi	27	0.013	0.009	0.014	0.476
O-Bak sampah	26	0.012	0.009	0.014	0.462
O-Ban bekas	26	0.012	0.009	0.014	0.448
I-Bak mandi	24	0.011	0.008	0.012	0.435
O-Bak air	23	0.011	0.008	0.012	0.422
I-Bak air	22	0.010	0.008	0.011	0.410
I-Tempayan plastik	21	0.010	0.007	0.011	0.399
O-Kaleng bekas	20	0.009	0.007	0.010	0.388
I-Bak mandi	19	0.009	0.007	0.010	0.378
I-Bak mandi	19	0.009	0.007	0.010	0.368
O-Ban bekas	16	0.007	0.006	0.008	0.358

Table 8.

Mynas neighbourhood in Iquitos, Peru. In this setting, the most productive containers were unused, or infrequently used, or abandoned large, rain-filled containers, mostly in the backs of the backyards. Only a few would need to be controlled to be prophylactic. No attention would need to be given to any of the indoor containers or backyard containers associated with water storage or clothes washing. This appears to be a clear example in which the targeting of a single class of container - the large, rain-filled, and abandoned container located towards the back of the property - would be sufficient. Under such circumstances, formative research and intervention development might be focused solely on these containers. Alternatively, inspectors with a search strategy only for these containers could inspect hundreds of houses per day, while ignoring the rest, and treat or eliminate only those found with large numbers of pupae.

Description	Number of pupae	Proportion of total	Pupae/person	Proportion of threshold	Cumulative pupae/person
DIVER	702	0.25	0.23	0.28	1.11
TQBJO	289	0.10	0.09	0.11	0.83
LLANT	208	0.07	0.07	0.08	0.72
DPLAS	109	0.04	0.03	0.04	0.64
CILIN	106	0.04	0.03	0.04	0.59
DPLAS	97	0.03	0.03	0.04	0.55
TQBJO	93	0.03	0.03	0.04	0.51
DPLAS	83	0.03	0.03	0.03	0.48
DIVER	71	0.03	0.02	0.03	0.45
DIVEO	68	0.02	0.02	0.03	0.42
TQBJO	62	0.02	0.02	0.02	0.39
DPLAS	48	0.02	0.02	0.02	0.37
DPLAS	39	0.01	0.01	0.02	0.35
TQBJO	36	0.01	0.01	0.01	0.33
CILIN	35	0.01	0.01	0.01	0.32
DPLAS	31	0.01	0.01	0.01	0.30
DPLAS	30	0.01	0.01	0.01	0.29
DILAT	30	0.01	0.01	0.01	0.28
CILIN	30	0.01	0.01	0.01	0.27
LLANT	27	0.01	0.01	0.01	0.26
CILIN	26	0.01	0.01	0.01	0.25
DPLAS	23	0.01	0.01	0.01	0.24
DPLAS	23	0.01	0.01	0.01	0.23
CILIN	22	0.01	0.01	0.01	0.22
LLANT	21	0.01	0.01	0.01	0.21
LLANT	20	0.01	0.01	0.01	0.20
DIVER	20	0.01	0.01	0.01	0.19
LLANT	16	0.01	0.01	0.01	0.19

Table 9.

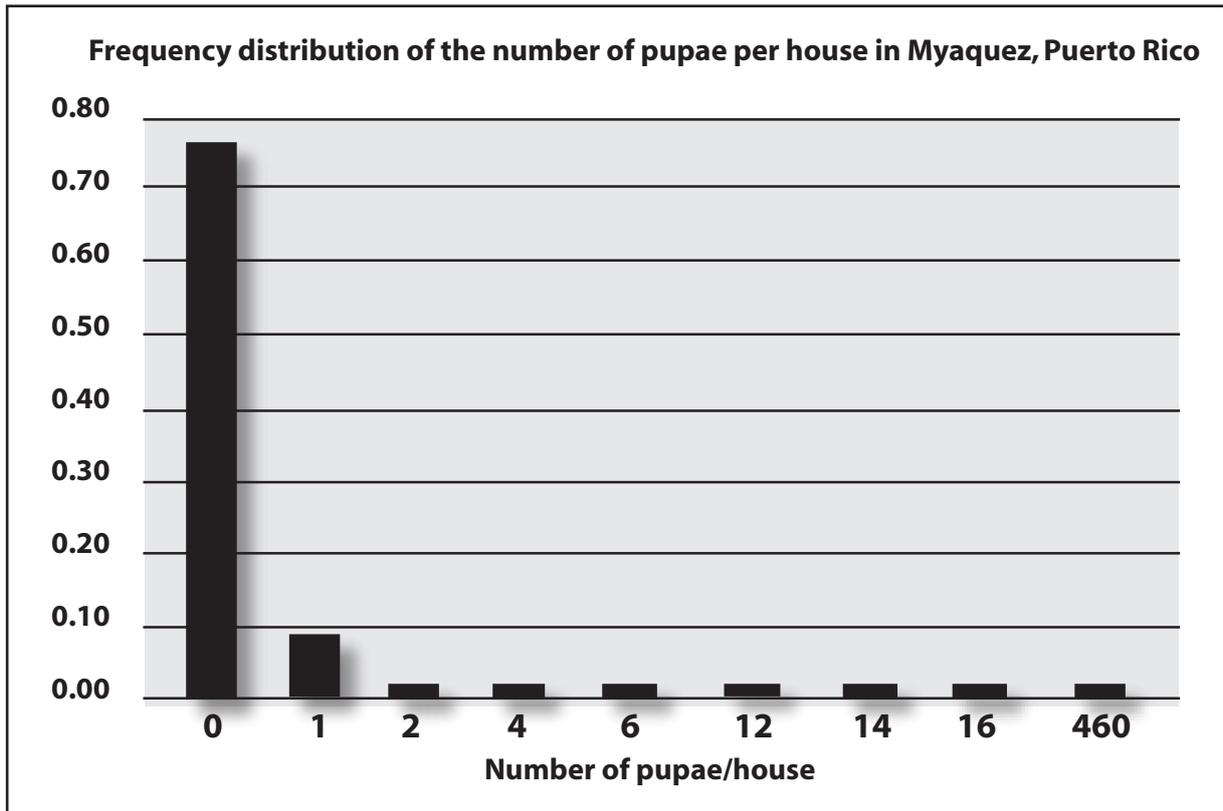
Survey results for Rio Cristal, Mayaguez, Puerto Rico. Of 46 houses surveyed, and 123 water-filled containers counted, only 16 had one or more *Ae. aegypti* pupae. The top two producers (a sink and a drum) were unused containers at one house. All major producers were situated outdoors. Elimination of three of the 16 positive containers would be prophylactic.

Description	Number of pupae	Proportion of total	Pupae/person	Proportion of threshold	Cumulative pupae/person
Sink	272	0.53	2.37	2.63	4.98
55-gallon drum	188	0.37	1.63	1.82	2.35
4-gallon bucket	14	0.03	0.12	0.14	0.53
Meter box	12	0.02	0.10	0.12	0.40
Meter box	8	0.02	0.07	0.08	0.28
1-gallon bucket	6	0.01	0.05	0.06	0.20
Meter box	4	0.01	0.03	0.04	0.14
Meter box	2	0.00	0.02	0.02	0.11
Saucer	2	0.00	0.02	0.02	0.09
Tyre	1	0.00	0.01	0.01	0.07
5-gallon bucket	1	0.00	0.01	0.01	0.06
Saucer	1	0.00	0.01	0.01	0.05
Meter box	1	0.00	0.01	0.01	0.04
Very small plant	1	0.00	0.01	0.01	0.03
Flower pot	1	0.00	0.01	0.01	0.02
Floor guttering	1	0.00	0.01	0.01	0.01
Tyre	0	0.00	0.00	0.00	0.00
Totals	515	1.00	4.48	4.98	

Figure 3 presents the frequency distribution of *Ae. aegypti* pupae per house in Mayaguez. Note that one house had 460 pupae. Whereas programme managers usually classify containers such that they can be targeted, perhaps an effort should be made to determine if particularly productive houses can be identified. Studies in the early 1980s by the author found, empirically, that peeling house paint and unmown lawns in New Orleans were associated with high populations of *Ae. aegypti*. This is also consistent with the observations of Tun-Lin et al on especially productive premises that gave rise to their PCI.⁵⁶

Figure 3.

Frequency distribution of *Ae. aegypti* pupae per house - note that one house had 460 pupae. Whereas programme managers usually classify containers such that they can be targeted, perhaps an effort to determine if particularly productive houses can be identified should be made. Studies in the early 1980s by the author, empirically found that peeling house paint and unmown lawns in New Orleans, Louisiana, were associated with high populations of *Ae. aegypti*.



How large do pupal/demographics need to be?

The question of just how many residences are required for an adequate survey is a topic of a number of projects currently under way. These projects involve repeated pupal/demographic surveys in thousands of residences in Peru, Indonesia, and Viet Nam. Published analyses should be available soon to provide guidance on sample size. In light of the information presented on the contagious distribution of pupae, this is obviously an important question.

Dengue early warning systems

A recent National Research Council (NRC) publication on the feasibility of developing practical and sustainable early warning systems (EWSs) for infectious diseases concluded that such systems could significantly improve control where mitigation methods were available.⁶⁰ Their primary value lies in the ability to focus scarce resources for control on those periods when epidemics are likely. Statistical EWSs have recently been developed in South-East Asia for dengue and DHF.⁶¹ Development was possible for three reasons: 1) an extensive time series on disease incidence was available; 2) dengue, being a vector-borne disease, is significantly influenced by weather; 3) in many sub-regions of South-East Asia, weather anomalies are significantly influenced by, and lag behind by several months, sea surface temperature (SST) anomalies.

Epidemic years were identified as those years when the total number of cases exceeds the mean plus one standard deviation of the period average. Using binary logistic regression, equations were developed that gave the probability of epidemics with three months lead time based on two variables: 1) SST anomalies as reported by the Japanese Meteorological Association (JMA); 2) previous cases. The physical area monitored by the JMA series spans a rectangular region between 90° and 150° E and 10° N to 10° S. SST anomalies subsequently affect air temperatures in the region, and air temperature in turn influences, among a host of other factors, the length of the gonotrophic cycle and the rapidity of viral dissemination within the vector, *Ae. aegypti*. So anomalous temperatures are strong correlates of transmission intensity, influencing the biting rate and the proportion of infectious females, though there is a delay before changes in incidence occur. Past cases are included as a correlate because they serve as a proxy for the types of virus circulating and the nature of human antibody types present—a function of previous dengue activity. Note that both of these variables are routinely collected and do not require additional information from dengue control operations in the area; this has important ramifications regarding practicality and sustainability by control specialists.

The NRC analysis was able to perfectly predict dengue in Yogyakarta but was inadequate for control operations in Bangkok. The authors were able to forecast with a three-month lead time in Yogyakarta because the region sits squarely within the JMA SST anomaly zone, and SST anomalies are highly correlated with subsequent surface air temperatures. Bangkok, being further north, has weaker coupling, resulting in delays of one to three months between SST anomalies and changes in case-reporting rates.

The author works actively with the South-East Asian and Western Pacific Regions of the World Health Organization (WHO), and with the countries of Thailand, Indonesia, Singapore, Viet Nam, and Indonesia, on dengue control, surveillance, and forecasting activities. In each of these countries, the principal person responsible for national dengue control programmes was asked what minimum lead time would be required to be useful for operational control programmes. The universal answer was a minimum of one month, while two or three months with less certainty would be useful as well. The notion is that a three-month forecast of likely epidemic conditions would lead to a *watch*, eliciting preparations for subsequent control, similar to the national weather service hurricane system of alerts - from watch to warning to temporal and spatial specification of evacuation and preparation. If the two-month forecast also indicated significant probability of an epidemic, then additional preparations could be made; the final decision to implement control would await confirmation with the one-month forecast.

The results presented in this preliminary work would only require a simple calculator, or preferably a personal computer using the derived equations, a spreadsheet, and the addition of monthly cases and JMA SST anomalies (available on the web) to make forecasts.

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