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VECTOR BREEDING SITE INFORMATION AS A TOOL FOR TRANSMISSION THRESHOLD ANALYSIS AND DENGUE RISK ASSESSMENT ON PENANG ISLAND, MALAYSIA

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ABSTRACT

Dengue is endemic in Malaysia and occurred mainly in the urban and suburban areas. While vaccine is currently under development, without immediate prospects for success, vector control remains the only viable method to prevent dengue transmission. The present study was conducted to assess and use pupae per person index as a tool to estimate the amount of suppression required in dengue interventions. Yearlong entomological surveys were carried out in urban-residential, urban-industrial, suburban and rural residential areas of the Penang Island. Houses were examined for the presence of water-holding containers and *Aedes* breeding. Pupae per person was used as indicator for determining dengue transmission threshold, assessing risk and the targeted control needed for source reduction efforts in a community. Pupae per person ranged between 0.53 to 1.09 for the study areas which indicated that the degree of suppression required to essentially eliminate the possibility of dengue transmission was estimated to be 33.3% (7 container classes), 61.5% (15 container classes), 65.5% (18 container classes) and 66.7% (13 container classes) of vector breeding containers in urban, urban industrial, suburban and rural residential areas respectively. Source reduction efforts targeting those container categories may yield significant and sustainable reductions in *Aedes* population in Penang Island.

Keywords: *Aedes*; target control; pupae; container; pupae per person; source reduction

ABSTRAK

Demam denggi merupakan endemik di Malaysia dan berlaku terutamanya di kawasan bandar dan pinggir bandar. Walaupun vaksin sedang dibangunkan, tanpa prospek kejayaan dalam masa terdekat, kawalan vektor kekal sebagai kaedah paling berkesan untuk mencegah penularan demam denggi. Kajian ini dijalankan untuk menilai dan menggunakan pupa per kapita sebagai alat untuk menganggarkan tahap pengurangan yang diperlukan dalam intervensi kawalan denggi. Tinjauan entomologi sepanjang tahun telah dilaksanakan di kawasan kediaman bandar, industri bandar, pinggir bandar, dan luar bandar di Pulau Pinang. Rumah-rumah diperiksa bagi mengenal pasti kehadiran bekas berisi air dan pembiakan *Aedes*. Pupa per kapita digunakan sebagai penunjuk untuk menentukan ambang penularan denggi, menilai risiko, dan sasaran kawalan yang diperlukan bagi usaha pengurangan sumber di sesuatu komuniti. Pupa per kapita berada dalam julat 0.53 hingga 1.09 bagi kawasan kajian, yang menunjukkan tahap pengurangan yang diperlukan untuk menghapuskan kemungkinan penularan denggi dianggarkan sebanyak 33.3% (7 kelas bekas), 61.5% (15 kelas bekas), 65.5% (18 kelas bekas) dan 66.7% (13 kelas bekas) bekas pembiakan vektor di kawasan kediaman bandar, industri bandar, pinggir bandar dan luar bandar masing-masing. Usaha pengurangan sumber yang menyasarkan kategori bekas ini berpotensi menghasilkan pengurangan populasi *Aedes* yang signifikan dan mampan di Pulau Pinang.

Kata Kunci: *Aedes*; kawalan sasaran; pupa; bekas; pupa perkapita; pengurangan sumber

INTRODUCTION

Dengue, Zika, and chikungunya are significant *Aedes*-borne diseases worldwide, posing serious public health challenges (Roiz et al. 2018; Roiz et al. 2024; Wilder-Smith et al. 2017). Efforts to prevent or reduce these diseases have primarily focused on the application of insecticides (Abad-Franch et al. 2017; Caetano & Yoneyama 2001; WHO 2010). Previously, this approach was highly effective in controlling mosquito populations (WHO 2014); however, recent reports highlight a concerning decline in insecticide efficacy in managing mosquito-borne diseases (van den Berg et al. 2021; Corbel & N'Guessan 2013; Dusfour & Chaney 2022; Patterson et al. 2016; Rivero et al. 2010; van den Meier et al. 2022). The widespread and prolonged use of insecticides has led to several disadvantages, including increasing resistance in mosquitoes to all four major classes of insecticides currently in use (Chanda et al. 2016; David et al. 2018; Dia et al. 2012), biodiversity loss, and adverse effects on animals (Lawler 2017) and humans (Peterson et al. 2006). Resistance to carbamates, organochlorines, organophosphates, and pyrethroids has been reported in *Aedes* mosquito populations (Ali et al. 2020; Elia-Amira et al. 2018; Elia-Amira et al. 2019). This resistance arises from reduced cuticular penetration, enhanced metabolic detoxification, target-site mutations, and behavioural avoidance, which collectively reduce insecticide efficacy by limiting uptake, degrading active ingredients, altering binding sites, or preventing exposure. Such mechanisms have been documented in *Aedes* populations in Malaysia and other regions.

This situation has necessitated the development and implementation of alternative vector control strategies that are safer for the environment and non-target organisms. Reduction by suppression rather than eradication of *Aedes* mosquito populations has been validated as an effective control strategy. Integrated control efforts, including sustained source reduction, SIT releases, or transgenic male deployments, have consistently reduced vector densities by up to 90% and correspondingly lowered human-vector contact (Carvalho et al. 2015; Humayra et al. 2025; Regis et al. 2008; Wu et el. 2024). Source reduction, an effort aimed at suppressing *Aedes* mosquito populations rather than eradication, has been demonstrated as an effective method for reducing vector densities and, subsequently, human-vector contact (Focks & Alexander 2006). Despite its effectiveness, this approach is labour-intensive and can be physically demanding for field operators. The workload can be significantly reduced if field personnel can identify specific container types that contribute the most to *Aedes* adult mosquito productivity and abundance. The ability to prioritise subsets of container types for intervention can enhance labour efficiency, reduce operational costs, and maximise reductions in adult mosquito populations.

Extensive breeding source reduction programmes have revealed that not all container types are equally significant, and the control of certain container types is far more critical than others (Focks 2003). Traditional *Aedes* indices, namely the House Index (HI), Container Index (CI), and Breteau Index (BI), have been widely used to assess mosquito infestation and the risk of dengue transmission. The relationship between these indices and actual dengue transmission thresholds has been investigated in many countries (Focks & Alexander 2006). However, recent studies have emphasized that these traditional indices have limited predictive value for dengue outbreaks. For example, Sihombing et al. (2020) and Wijayanti et al. (2016) reported weak correlations between HI, CI, and BI values and actual dengue incidence in Indonesia. Similarly, in Singapore, Liew et al. (2019) demonstrated that dengue outbreaks continued to occur despite low House Index values, prompting the development of a new entomological indicator the *Aedes aegypti* Breeding Percentage Index which showed stronger correlation with dengue case burden. These findings suggest a shift is needed towards more sensitive and spatially relevant indices in mosquito surveillance and control.

One of the limitations of traditional larval surveillance is the inclusion of pupae within the total count of immature stages, where they are treated equivalently to larvae. However, pupae are more epidemiologically significant as they represent the final developmental stage before adult emergence. Among all immature stages, the pupal stage of *Ae. albopictus* exhibited the lowest mortality under field conditions (Nur Aida et al. 2008). Higher pupal survival could also lead to an increase in mosquito population density (Nur Aida et al. 2011). Focks & Chadee (1997) proposed the pupal index, an estimate of the number of ‘pupae per person’ as a preferred indicator for dengue risk assessment, as it more accurately reflects adult mosquito production before emergence. This pupal-based approach overcomes key limitations of *Stegomyia* indices. Recent evidence continues to support its utility: studies in Argentina confirm that pupal indices better predict adult *Aedes aegypti* abundance than larval-based indices (Garelli et al. 2009), and the Camino Verde community trial in Mexico demonstrates that pupae-per-person and pupae-per-household indices remained stable across seasons and corresponded with effective dengue reduction efforts (Andersson et al. 2017).

By focusing on pupal surveillance, control efforts can be better directed toward the most productive breeding sites, thereby achieving more efficient and sustainable vector control outcomes. Recent studies also support this idea. In Cambodia, Seng et al. (2009) found that the pupal index showed better results for vector control compared to larval counts. A study in Mexico by Garelli et al. (2009) showed that pupal counts stayed stable across seasons and matched well with dengue cases after control work. In India and Sri Lanka, researchers also found that larval indices like House Index or Breteau Index did not clearly show dengue risk, but pupal indices gave more reliable results (Udayanga et al. 2020; Sharma et al. 2024).

The present study was conducted to assess the productivity of *Aedes* mosquitoes in water-holding containers of various sizes using pupae as the primary indicator. The transmission threshold proposed by Focks et al. (2000) was utilised to evaluate dengue risk based on the *pupae per person index*. The study also aimed to identify container types contributing most significantly to pupal production and to estimate the reduction required to bring transmission risk below the threshold. These assessments were performed in four dengue-endemic areas within the Southwest District of Penang Island, Malaysia.

MATERIALS AND METHODS

Study Sites

The study was conducted in four dengue-endemic areas within the Southwest District of Penang Island from January to December 2009. The selected sites represented different geographical settings, namely Pantai Jerjak (urban residential area), Bayan Lepas (urban residential/industrial area), Batu Maung (suburban residential area), and Balik Pulau (rural area). Sampling was carried out throughout the one-year period.

Field Surveys

Field entomological surveys were conducted once per month in each study area between 0900 h and 1500 h. This period was chosen for logistical convenience and better access to households. In each study area, sixty houses were visited monthly, amounting to a total of 720 houses surveyed per area over the year-long study. Three collection teams, consisting of trained personnel, inspected households and their surrounding environments for the presence of water-holding containers. The surveys included both indoor and peri-domestic areas, where all water-holding containers of varying types and sizes were examined for the presence of *Aedes* immatures (larvae and pupae). In addition, data on household occupancy were also recorded.

For each container found with *Aedes* immatures, information on the container type, container size, and location (indoor or outdoor) was documented. A photograph of each container was also taken. Immature stages of mosquitoes were collected using appropriate methods depending on container size and water conditions. Pipettes were used for small volumes of water and sieves were used for larger water bodies. While water from some containers was poured into individual labelled plastic bags.

Each container was labelled with relevant information, including container type, size, and identification number. All collected samples were transported to the laboratory at the School of Biological Sciences, Universiti Sains Malaysia, for further analysis.

Identification of Mosquitoes

Pupae were allowed to emerge into adults for species identification. First and second instar larvae were reared to the third and fourth instar stages to facilitate identification. Third and fourth instar larvae, as well as adult mosquitoes that emerged from pupae, were directly identified to the species level under a dissecting microscope (Olympus CX41, Olympus, Tokyo, Japan). Species identification was performed using morphological identification keys provided by Rueda (2008).

Data Analyses

Only container positive with pupae were used in data analysis. Analysis of pupae per person and transmission threshold for assessing risk and to determine the targeted control operation needed for source reduction efforts in a community were done on the spreadsheets in Microsoft Office Excel 365 for windows by following (Focks et al. 2000).

To estimate the transmission threshold at 28.8°C for 33% seroprevalence, a cubic spline interpolation model was applied to the data from Focks et al. (2000) (Table 1). The model was constructed using R software (version 4.3.1; R Core Team 2023). This method captures the smooth, non-linear decline in threshold values without forcing unrealistic trends. This approach is particularly appropriate given the small number of data points.

Table 1. Transmission threshold by initial seroprevalence of antibody of 0%, 33% and 67% (Focks et al. 2000)

Temperature (°C)	**Transmission threshold by initial seroprevalence of antibody**		
	0%	**33%**	**67%**
22	7.13	10.7	23.32
24	2.2	3.47	7.11
26	1.05	1.55	3.41
28	0.42	0.61	1.27
30	0.1	0.15	0.3
32	0.06	0.09	0.16

Thresholds estimated using the DENSiM model (Focks et al. 2000) based on seroprevalence (0–67%), ambient temperature, and frequency of viral introductions. Seroprevalence is proportion of individuals testing positive for dengue antibodies at a given time.

Based on average temperature for the hottest month (28.8°C) and assuming a seroprevalence rate of 33%, the estimate of the transmission threshold in Penang Island is 0.36 (interpolation of Table 1). The transmission threshold of 0.36 means, pupae per person observed value (PP) in the field conditions cannot exceed 0.36. The ratio of PP to transmission threshold (pupae per person estimated) must be 1.0 or less. If the value surpasses the transmission threshold, therefore the risk of dengue or other *Aedes*-borne virus (transmission will be increased and the ratio must be lowered until value of 1.0 ratio is achieved).

RESULTS

The results of this study highlight the magnitude of *Aedes* pupal productivity and the reductions required to lower dengue transmission risk in the four study areas: Pantai Jerjak, Bayan Lepas, Batu Maung, and Balik Pulau. By comparing the observed PP with the transmission threshold of 0.36 pupae per person, the study provides a clear estimate of the interventions necessary to control dengue risk effectively (Table 2).

In Pantai Jerjak, a total of 3,198 residents from 720 households were surveyed, of which 195 houses were positive for *Aedes* breeding. The observed PP for the area was 0.53, exceeding the transmission threshold of 0.36. This indicates that approximately 33.3% of the breeding containers, or five out of every fifteen, would need to be eliminated to reduce the vector population to a level below the transmission threshold (Table 2). A total of 218 pupae-inhabited containers were identified, categorised into 43 distinct container classes (Table 3). Among these, seven container classes contributed disproportionately to pupal productivity: buckets, sheets, tyres, container covers/lids, assorted plastic containers, flower and plant pot and paint mix container. Buckets alone accounted for 26.3% of the total pupae collected in Pantai Jerjak, making them the most productive container class. Eliminating these seven container classes would significantly reduce pupal abundance, lowering the risk of dengue transmission.

Table 2. Observed *Aedes* Pupae per Person, Transmission thresholds, and Estimated required reductions in dengue endemic areas of southwest Penang Island

		***Aedes* pupae**				**Estimated reduction required for control**		
Location	**Temp (°C)**	**Total**	**Pupa per person**	**Transmission threshold**	**Ratio (Proportion of threshold)**	**Example number of container**	**Number of containers to eliminate**	**%**
All sites Combined	28.8	10217	0.89	0.36	2.47	25	15	60.0
Pantai Jerjak	28.8	1702	0.53	0.36	1.47	14	4	33.3
Bayan Lepas	28.8	2840	0.95	0.36	2.64	26	16	61.5
Batu Maung	28.8	2901	1.04	0.36	2.89	29	19	65.5
Balik Pulau	28.8	2774	1.09	0.36	3.03	30	20	66.7

Temp: average temperature during the hottest month.
Total: total number of pupae observed in each area.
Pupae per person: average number of *Aedes* pupae per person observed during surveys.
Transmission threshold: the estimated number *Aedes* pupae per person that will preclude epidemics transmission from Focks et al. (2000).
Ratio (Proportion of threshold): the ratio of observed pupae per person and seroprevalence specific transmission threshold.
%: the degree of reduction in number of containers that needs to be controlled or eliminated necessary to reduce the observed field level below threshold.

Table 3. Survey results from Pantai Jerjak (January–December 2009) with a dengue transmission threshold of 0.36. Among 218 pupae-positive containers (43 types) linked to 3,198 residents, the seven most productive container classes (in bold) are highlighted for targeted control to reduce dengue risk

Container Description	**Number of Containers**	**Total Pupae**	**Proportion of Total Pupa**	**Pupa/Person**	**Proportion of Threshold**	**Cumulative Proportion of Threshold**
Bucket	**32**	**448**	**26.32**	**0.14**	**0.33**	**1.24**
Sheet	**10**	**276**	**16.22**	**0.09**	**0.20**	**0.91**
Tyre	**17**	**178**	**10.46**	**0.06**	**0.13**	**0.71**
Cover or lid	**18**	**158**	**9.28**	**0.05**	**0.11**	**0.58**
Plastic container	**10**	**100**	**5.88**	**0.03**	**0.07**	**0.47**
Flower or plant pot	**8**	**52**	**3.06**	**0.02**	**0.04**	**0.39**
Paint mix cont	**7**	**50**	**2.94**	**0.02**	**0.04**	**0.36**

Bottle or jar	11	37	2.17	0.01	0.03	0.32
Sink	8	26	1.53	0.01	0.02	0.29
Cement tank	8	25	1.47	0.01	0.02	0.27
Tin or can	5	25	1.47	0.01	0.02	0.26
Small pothole or water puddle	5	25	1.47	0.01	0.02	0.24
Bucket's rim	3	24	1.41	0.01	0.02	0.22
Tyre's rim	3	24	1.41	0.01	0.02	0.20
Vehicle parts	5	23	1.35	0.01	0.02	0.18
Ice cream container	3	20	1.18	0.01	0.01	0.17
Cup or bowl	5	19	1.12	0.01	0.01	0.15
Road divider cone	2	18	1.06	0.01	0.01	0.14
Drum	5	15	0.88	0.00	0.01	0.13
Furniture parts	3	15	0.88	0.00	0.01	0.12
Small container	6	15	0.88	0.00	0.01	0.10
Hole on wood	3	15	0.88	0.00	0.01	0.09
Animal food or drink Container	4	14	0.82	0.00	0.01	0.08
Waste container	2	12	0.71	0.00	0.01	0.07
Basin	5	9	0.53	0.00	0.01	0.06
Bucket (upturned)	3	9	0.53	0.00	0.01	0.06
Basket	3	9	0.53	0.00	0.01	0.05
Plant watering pot	3	9	0.53	0.00	0.01	0.04
Plastic tank	2	8	0.47	0.00	0.01	0.04
Food packaging)	1	6	0.35	0.00	0.00	0.03
Shoe, sandal or boot	2	6	0.35	0.00	0.00	0.03
Concrete hole	1	5	0.29	0.00	0.00	0.02
Pipe	1	4	0.24	0.00	0.00	0.02
Jug or kettle	2	4	0.24	0.00	0.00	0.02
Electrical appliance	1	4	0.24	0.00	0.00	0.01

Toilet bowl	1	3	0.18	0.00	0.00	0.01
Tin's rim	3	3	0.18	0.00	0.00	0.01
Aquarium	2	2	0.12	0.00	0.00	0.01
Plastic bag	1	2	0.12	0.00	0.00	0.01
Cable roller's rim	1	2	0.12	0.00	0.00	0.00
Tree hole	1	1	0.06	0.00	0.00	0.00
Tray	1	1	0.06	0.00	0.00	0.00
Letter box	1	1	0.06	0.00	0.00	0.00
Total	**218**	**1702**	**100**			

In Bayan Lepas, surveys were conducted across 720 households, covering a total human population of 2,874 residents. Out of the surveyed households, 212 houses were found positive for *Aedes* breeding, indicating significant mosquito infestation in this urban-industrial area. The observed pupae per person (PP) in Bayan Lepas was 0.95, which exceeds the transmission threshold of 0.36 pupae per person. To reduce dengue transmission risk in this area, approximately 61.5% of breeding containers, or sixteen out of every twenty-six containers, would need to be eliminated (Table 2).

A total of 245 pupae-inhabited containers were identified in Bayan Lepas, representing 45 distinct container classes (Table 4). Among these, the most productive container classes contributing to the majority of pupae were buckets, tyres, container lids/covers, plastic tanks and assorted plastic containers. The results revealed that buckets was also the single most productive container class in Bayan Lepas, contributing 22.4% of the total pupae collected. Tyres followed closely as significant contributors, accounting for 14.6% of pupae, reflecting the industrial and peri-domestic nature of this area where discarded tyres are abundant. Other containers such as plastic tanks, assorted plastic containers, and container covers/lids collectively contributed smaller but notable proportions to the total pupal count.

Table 4. Survey results from Bayan Lepas (January–December 2009) with a dengue transmission threshold of 0.36. Among 249 pupae-positive containers (45 classes) linked to 3,000 residents, the 15 most productive container classes (in bold) are highlighted for targeted control to reduce dengue risk

Container Description	No. of Container	Total Pupae	Proportion of Total Pupa	Pupae/ Person	Proportion of Threshold	Cumulative Proportion of Threshold
Bucket	**38**	**691**	**24.33**	**0.23**	**0.54**	**2.20**
Small pothole/water Puddle	**2**	**356**	**12.54**	**0.12**	**0.28**	**1.67**
Cover/lid	**14**	**192**	**6.76**	**0.06**	**0.15**	**1.39**
Tyre	**22**	**146**	**5.14**	**0.05**	**0.11**	**1.24**
Plastic container	**11**	**141**	**4.96**	**0.05**	**0.11**	**1.13**
Waste container	**1**	**120**	**4.23**	**0.04**	**0.09**	**1.02**
Sheet	**10**	**113**	**3.98**	**0.04**	**0.09**	**0.93**
Drum	**12**	**111**	**3.91**	**0.04**	**0.09**	**0.84**
Aquarium	**5**	**111**	**3.91**	**0.04**	**0.09**	**0.75**
Flower/plant pot	**8**	**95**	**3.35**	**0.03**	**0.07**	**0.67**
Cement tank	**12**	**88**	**3.10**	**0.03**	**0.07**	**0.59**
Basin	**5**	**85**	**2.99**	**0.03**	**0.07**	**0.52**
Crate hole	**1**	**69**	**2.43**	**0.02**	**0.05**	**0.46**
Tin/can	**23**	**54**	**1.90**	**0.02**	**0.04**	**0.40**
Cup/bowl	**10**	**47**	**1.65**	**0.02**	**0.04**	**0.36**
Animal food/drink container	7	45	1.58	0.02	0.03	0.33
Flush tank	2	42	1.48	0.01	0.03	0.29
Ice cream container	2	34	1.20	0.01	0.03	0.26
Plastic tank	2	31	1.09	0.01	0.02	0.23

Bottle/jar	12	27	0.95	0.01	0.02	0.21
Earthen jar	1	20	0.70	0.01	0.02	0.19
Bucket's rim	9	19	0.67	0.01	0.01	0.17
Plastic box	2	19	0.67	0.01	0.01	0.16
Flower vase	2	18	0.63	0.01	0.01	0.14
Paint mix container	2	16	0.56	0.01	0.01	0.13
Small container	1	15	0.53	0.01	0.01	0.12
Hole on wood	3	15	0.53	0.01	0.01	0.10
Coconut shell	2	14	0.49	0.00	0.01	0.09
Food packaging	3	11	0.39	0.00	0.01	0.08
Door/roof	3	11	0.39	0.00	0.01	0.07
Washing machine	1	9	0.32	0.00	0.01	0.07
Tile roof	1	9	0.32	0.00	0.01	0.06
Pipe	2	8	0.28	0.00	0.01	0.05
Cooking pot	2	8	0.28	0.00	0.01	0.04
Tray	1	8	0.28	0.00	0.01	0.04
Vehicle parts	2	7	0.25	0.00	0.01	0.03
Basket	2	6	0.21	0.00	0.00	0.03
Toolbox	1	6	0.21	0.00	0.00	0.02
Plastic bag	1	5	0.18	0.00	0.00	0.02
Safety helmet	1	4	0.14	0.00	0.00	0.01
Bucket (upturned)	1	4	0.14	0.00	0.00	0.01
Plant watering pot	4	4	0.14	0.00	0.00	0.01
Plant parts (leaf, axils)	1	3	0.11	0.00	0.00	0.00
Cable roller's rim	1	2	0.07	0.00	0.00	0.00
Shoe/sandal/boot	1	1	0.04	0.00	0.00	0.00
Total	**249**	**2840**	**100.00**			

The findings suggest that by focusing efforts on eliminating or managing key container types especially those common in urban (e.g., tyres, buckets) and rural (e.g., coconut shells) areas a significant reduction in *Aedes* pupal productivity can be achieved. This targeted intervention approach would optimise resource allocation and control efforts, bringing the PP below the dengue transmission threshold.

The results also highlight the influence of urban-industrial settings on *Aedes* breeding. The presence of artificial containers, such as tanks and lids, combined with the improper disposal of tyres, underscores the importance of community awareness and active involvement in managing such high-risk breeding sites. Elimination of fifteen container classes from the field is required to lower dengue risk below the threshold value of 0.36.

In Batu Maung, surveys encompassed 2,796 residents across 720 households, of which 238 houses were positive for *Aedes* breeding. The observed PP in this area was 2.89, far exceeding the threshold value of 0.36 (Table 2). To bring the risk below the transmission threshold, approximately 65.5% of the breeding containers, or nineteen out of every twenty-nine, would need to be eliminated. A total of 261 pupae-inhabited containers were identified, representing 45 container classes (Table 5). The most productive container types in Batu Maung included buckets, sheets, aquariums/fish tanks, assorted plastic containers, and plastic tanks. Buckets again emerged as the primary contributor, accounting for 23.2% of the total pupae collected. The remaining productive containers, such as tyres, container lids, and tins/cans, also played a significant role. This suggests that targeted elimination of these highly productive container types would significantly reduce pupal abundance in Batu Maung, contributing to a lower risk of dengue transmission. A total of eighteen container classes must be removed from the Batu Maung to reduce dengue risk to below the 0.36 threshold value.

Table 5. Survey results from Batu Maung (January–December 2009) with a dengue transmission threshold of 0.36 pupae per person. Among 261 pupae-positive containers (45 classes) linked to 2,796 residents, the 18 most productive container classes (in bold) are highlighted for targeted control to reduce dengue risk

Container Description	No. of Container	Total Pupae	Proportion of Total Pupa	Pupae/ Person	Proportion of Threshold	Cumulative Proportion of Threshold
Bucket	**52**	**673**	**23.2**	**0.24**	**0.56**	**2.41**
Sheet	**18**	**268**	**9.24**	**0.1**	**0.22**	**1.85**
Aquarium or fish tank	**3**	**218**	**7.51**	**0.08**	**0.18**	**1.63**
Plastic container	**11**	**215**	**7.41**	**0.08**	**0.18**	**1.45**
Plastic tank	**3**	**174**	**6**	**0.06**	**0.14**	**1.27**
Tyre	**15**	**115**	**3.96**	**0.04**	**0.1**	**1.13**
Cover or lid	**15**	**103**	**3.55**	**0.04**	**0.09**	**1.03**
Ice cream container	**4**	**94**	**3.24**	**0.03**	**0.08**	**0.94**
Tin or can	**12**	**90**	**3.1**	**0.03**	**0.07**	**0.87**
Flush tank	**1**	**89**	**3.07**	**0.03**	**0.07**	**0.79**
Flower or plant pot	**12**	**69**	**2.38**	**0.02**	**0.06**	**0.72**
Coconut shell	**9**	**64**	**2.21**	**0.02**	**0.05**	**0.66**
Cement tank	**11**	**63**	**2.17**	**0.02**	**0.05**	**0.61**
Basin	**4**	**59**	**2.03**	**0.02**	**0.05**	**0.55**
Tray	**1**	**58**	**2**	**0.02**	**0.05**	**0.5**
Bottle or jar	**10**	**57**	**1.96**	**0.02**	**0.05**	**0.46**
Bucket's rim	**15**	**52**	**1.79**	**0.02**	**0.04**	**0.41**
Animal food or drink container	**7**	**51**	**1.76**	**0.02**	**0.04**	**0.37**
Plastic bag	3	45	1.55	0.02	0.04	0.32
Plastic box	1	35	1.21	0.01	0.03	0.29
Shoe, sandal or boot	4	35	1.21	0.01	0.03	0.26
Earthen jar	4	33	1.14	0.01	0.03	0.23
Pipe	4	27	0.93	0.01	0.02	0.2
Cup or bowl	4	27	0.93	0.01	0.02	0.18
Food packaging	1	24	0.83	0.01	0.02	0.16
Drum	3	22	0.76	0.01	0.02	0.14
Furniture parts	3	18	0.62	0.01	0.01	0.12
Basket	4	18	0.62	0.01	0.01	0.1

Bucket (upturned)	4	16	0.55	0.01	0.01	0.09
Paint mix container	2	16	0.55	0.01	0.01	0.07
Portable swimming pool	1	11	0.38	0	0.01	0.06
Washing machine	1	9	0.31	0	0.01	0.05
Toolbox	2	8	0.28	0	0.01	0.04
Raincoat	1	8	0.28	0	0.01	0.04
Crate hole	5	7	0.24	0	0.01	0.03
Tree hole	2	6	0.21	0	0	0.02
Toilet bowl	1	5	0.17	0	0	0.02
Jug/kettle	1	4	0.14	0	0	0.02
Door/roof	1	3	0.1	0	0	0.01
Ashtray	1	3	0.1	0	0	0.01
Small pothole/water puddle	1	2	0.07	0	0	0.01
Fridge drawer	1	2	0.07	0	0	0.01
Home cleaning equipment	1	2	0.07	0	0	0
Electrical appliance	1	2	0.07	0	0	0
Safety helmet	1	1	0.03	0	0	0
Total	**261**	**2901**	**100**	**1.04**	**2.41**	

In Balik Pulau, a total of 2,546 residents across 720 households were surveyed, and 201 houses were positive for *Aedes* breeding. The observed PP was 3.03, indicating a transmission risk far above the threshold (Table 2). To reduce the vector population to a safe level, 61.5% of the breeding containers, or twenty out of every thirty, would need to be eliminated. A total of 304 pupae-inhabited containers were identified, representing 33 container classes (Table 6). In contrast to the urban and suburban areas, Balik Pulau exhibited a mix of artificial and natural container types. The most productive container classes included buckets, sheets, coconut shells, cement tanks, and tins/cans. Notably, coconut shells contributed significantly to pupal productivity due to their abundance in this rural setting, making them an important target for source reduction. Buckets, similar to the other areas, remained the most productive container class, contributing 16.2% of the total pupae collected. Removing thirteen container classes from the field is necessary to bring the dengue risk below the threshold value of 0.36.

Table 6. Survey results from Balik Pulau (January–December 2009) with a dengue transmission threshold of 0.36 pupae per person. Among 304 pupae-positive containers (33 classes) linked to 2,546 residents, the 13 most productive container classes (in bold) are highlighted for targeted control to reduce dengue risk

Container Description	No. of Container	Total Pupae	Proportion of Total Pupa	Pupae/ Person	Proportion of Threshold	Cumulative Proportion of Threshold
Bucket	**33**	**448**	**16.15**	**0.18**	**0.41**	**2.53**
Sheet	**29**	**444**	**16.01**	**0.17**	**0.41**	**2.12**
Cement tank	**20**	**375**	**13.52**	**0.15**	**0.34**	**1.72**
Coconut shell	**14**	**188**	**6.78**	**0.07**	**0.17**	**1.38**
Tin or can	**28**	**154**	**5.55**	**0.06**	**0.14**	**1.20**

Tyre	**24**	**148**	**5.34**	**0.06**	**0.14**	**1.06**
Basin	**3**	**144**	**5.19**	**0.06**	**0.13**	**0.93**
Plastic container	**16**	**108**	**3.89**	**0.04**	**0.10**	**0.80**
Flower or plant pot	**17**	**108**	**3.89**	**0.04**	**0.10**	**0.70**
Cover or lid	**8**	**87**	**3.14**	**0.03**	**0.08**	**0.60**
Bucket's rim	**25**	**68**	**2.45**	**0.03**	**0.06**	**0.52**
Fridge drawer	**6**	**67**	**2.42**	**0.03**	**0.06**	**0.46**
Bottle or jar	**10**	**64**	**2.31**	**0.03**	**0.06**	**0.40**
Ice cream container	7	64	2.31	0.03	0.06	0.34
Shoe, sandal or boot	7	43	1.55	0.02	0.04	0.28
Earthen jar	6	40	1.44	0.02	0.04	0.24
Jug or kettle	4	38	1.37	0.01	0.03	0.20
Animal food or drink bowl	5	32	1.15	0.01	0.03	0.17
Bucket (upturned)	5	29	1.05	0.01	0.03	0.14
Sink	5	26	0.94	0.01	0.02	0.11
Cup or bowl	9	19	0.68	0.01	0.02	0.09
Plastic bag	3	19	0.68	0.01	0.02	0.07
Door or roof	1	16	0.58	0.01	0.01	0.06
Polystyrene food container	3	12	0.43	0.00	0.01	0.04
Drum	3	12	0.43	0.00	0.01	0.03
Food packaging	3	9	0.32	0.00	0.01	0.02
Plant parts (leaf, apex)	2	3	0.11	0.00	0.00	0.01
Vehicle parts	2	3	0.11	0.00	0.00	0.01
Small container	2	2	0.07	0.00	0.00	0.01
Safety helmet	1	1	0.04	0.00	0.00	0.00
Plastic tank	1	1	0.04	0.00	0.00	0.00
Furniture parts	1	1	0.04	0.00	0.00	0.00
Toolbox	1	1	0.04	0.00	0.00	0.00
Total	**304**	**2774**	**100.00**			

When combining all study sites, the overall observed PP was 0.89, which is approximately almost three times the transmission threshold. To achieve an effective reduction in dengue risk across all areas combined, it was estimated that 60.0% of the breeding containers, or fifteen out of every twenty-five, would need to be eliminated. Across the study areas, buckets consistently ranked as the most productive container class, contributing the largest proportion of pupae in all three settings. Other key contributors varied based on geographical and socio-ecological factors. In urban Pantai Jerjak and suburban Batu Maung, artificial containers such as sheets, tyres, plastic tanks, and aquariums were major breeding sites, whereas in rural Balik Pulau, natural containers like coconut shells also played a significant role.

The study demonstrates the clumped nature of *Aedes* pupal distribution, with only a small number of container classes accounting for the majority of pupal production. By targeting the most productive containers, control efforts can be optimised to achieve maximum reductions in *Aedes* populations. These findings provide a practical framework for targeted source reduction interventions, which are essential for lowering dengue transmission risk in dengue-endemic areas like the Southwest District of Penang Island.

DISCUSSION

The findings of this study provide significant information on the *Aedes* pupal productivity across four distinct geographical settings: urban (Pantai Jerjak), urban residential/industrial area (Bayan Lepas), suburban (Batu Maung), and rural (Balik Pulau) within the Southwest District of Penang Island. By using the pupae per person (PP) in combination with a transmission threshold (0.36 pupae per person), this study demonstrates the importance of targeted interventions to reduce *Aedes* populations and mitigate dengue transmission risk.

The results indicate that pupal productivity was unevenly distributed across water-holding containers, supporting the findings of earlier studies (Focks 2003; Focks et al. 2000). A small number of container types consistently accounted for the majority of pupal production, while the majority of containers yielded few or no pupae. This clumped distribution is a well-documented phenomenon in *Aedes* ecology, where a few highly productive breeding sites play a disproportionately significant role in sustaining adult mosquito populations. For example, buckets were consistently identified as the most productive container class across all study sites, contributing 26.3% of pupae in Pantai Jerjak, 22.4% in Bayan Lepas, 23.2% in Batu Maung, and 16.2% in Balik Pulau. Other highly productive containers, such as sheets, tyres, and container lids, also played a critical role, varying slightly depending on the area's geographical and socio-ecological characteristics.

The differences in pupal productivity among container types show why localised control strategies are needed. On Penang Island, most residents live in modern houses or apartments with access to piped water. People take showers, and water supply disruption is rare. Because of this, most residents do not store water for daily use. Water tanks are mostly seen in rural or suburban areas where water storage is still a common practice. This water use pattern affects the types of mosquito breeding containers found in different areas.

In urban areas like Pantai Jerjak, artificial containers such as buckets, plastic sheets, and tyres were the main sources of pupae, showing the effect of human activity and poor disposal of household waste. In Bayan Lepas, a more industrial area, tyres were also major contributors (14.6% of total pupae), probably due to the presence of discarded tyres in and around industrial zones. Other productive containers here included plastic tanks, buckets, various plastic containers, and lids.

In the suburban area of Batu Maung, a wider variety of breeding sites were found, such as aquariums, plastic tanks, and cisterns, showing more diverse water storage habits. Meanwhile, in the rural area of Balik Pulau, both natural containers like coconut shells and man-made containers such as cement tanks and buckets contributed to mosquito breeding. The large number of coconut shells in rural areas shows the need to also consider natural habitats when planning mosquito control strategies.

Similar patterns were reported in other countries. In Tripura, India, discarded tyres were the most productive breeding containers in urban and semi-urban households, more than other containers like plastic buckets or drums (Thakuria et al. 2024). In Dhaka, Bangladesh, most pupae came from outdoor plastic containers like drums, buckets, and flower tubs, especially those larger than 50 litres (Islam et al. 2019). A study in Cambodia also showed that water storage jars and concrete tanks produced over 90% of pupae, while tyres played a smaller but still important role (Seng et al. 2022).

These findings clearly show that both domestic and industrial containers contribute to mosquito breeding. Therefore, household-level action, such as removing unused containers, and better waste management, especially in industrial areas, are both important to reduce *Aedes* pupal productivity and prevent dengue.

The observed pupae per person (PP) and transmission threshold ratio varied significantly across the four study areas. Balik Pulau recorded the highest ratio (3.03), followed by Batu Maung (2.89), Bayan Lepas (2.64) and Pantai Jerjak (1.47), all of which exceeded the value of 1.0. These ratio values indicate a high risk of dengue transmission in all four areas, particularly in Bayan Lepas, Balik Pulau and Batu Maung, where more than 50% of breeding containers would need to be eliminated to reduce transmission risk below the threshold. The differences in the threshold ratio values among the study areas can be attributed to a combination of environmental factors, water storage practices, garbage disposal, and socio-economic conditions, which influence the availability and persistence of productive breeding sites.

Traditional *Aedes* indices, such as the House Index (HI), Container Index (CI), and Breteau Index (BI), have long been used as surveillance tools for dengue vector control. However, these indices have limitations, as they do not account for the productivity of individual containers or provide an accurate measure of adult mosquito emergence. In contrast, the pupal survey approach, specifically the pupae per person, offers a more reliable indicator of dengue transmission risk because pupae represent the final developmental stage before adult emergence. As such, PP directly correlates with the adult mosquito population, which is the primary vector responsible for dengue virus transmission.

The study also highlights the practical advantages of using PP for guiding targeted source reduction efforts. By focusing on the most productive container classes, control programmes can achieve significant reductions in *Aedes* populations while optimising resource allocation. For example, eliminating buckets alone would result in a 26.3% reduction in pupal production in Pantai Jerjak, a 22.4% reduction in Bayan Lepas, a 23.2% reduction in Batu Maung, and a 16.2% reduction in Balik Pulau. This targeted approach contrasts with conventional blanket elimination strategies, which are often labour-intensive, costly, and less effective. By identifying and prioritising the elimination of specific container types, control programmes can maximise their impact while minimising operational costs and effort.

In establishing integrated community-based vector control programmes, it is essential to account for both the larval ecology and the sociological importance of the container habitats, alongside identifying control measures that are most appropriate for their management (Nathan & Knudsen 1991). Geographical and socio-ecological differences must also be considered when designing control interventions. Urban and suburban areas, where artificial containers are the primary breeding habitats, require community engagement and education to promote proper waste management and water storage practices. Residents should be encouraged to eliminate or manage high-risk containers, such as buckets and tyres, to prevent *Aedes* breeding. In rural areas like Balik Pulau, additional efforts are needed to address natural containers such as coconut shells, which are often overlooked in conventional control programmes. Community participation and awareness campaigns tailored to local conditions are essential for the success of source reduction efforts.

The findings of this study are consistent with previous research (Focks & Chadee 1997; Tun-Lin et al. 1996), which emphasised the importance of pupal surveys as a more accurate and practical tool for dengue vector surveillance and risk assessment. Unlike traditional indices, PP not only provides an estimation of dengue transmission risk but also helps determine the magnitude of intervention required to reduce vector populations below transmission thresholds. This approach allows for evidence-based decision-making, enabling control programmes to prioritise resources and focus efforts where they are most needed.

Moreover, the study demonstrates that the risk of dengue transmission is not solely determined by *Aedes* indices but also by the introduction and circulation of the dengue virus within a community (Silver 2008). However, the use of PP in conjunction with transmission thresholds provides a valuable framework for assessing vector infestation severity, identifying critical breeding habitats, and designing effective intervention strategies. By combining pupal surveys with targeted source reduction efforts, control programmes can achieve sustainable reductions in *Aedes* populations and dengue transmission risk.

CONCLUSION

In conclusion, this study shows the importance of the pupae per person as a reliable and practical tool for dengue vector monitoring and control. The results highlight the clumped nature of *Aedes* pupal distribution, the key role of certain container types in pupal productivity, and the need for area-specific, targeted actions. By focusing on the most productive containers, such as buckets, tyres, and coconut shells, dengue control programmes can better allocate resources, reduce costs, and achieve large reductions in *Aedes* populations. This approach offers a strong framework for evidence-based dengue control strategies suited to the unique conditions of each area. A limitation is that dengue transmission threshold was derived from (Focks et al. 2000) using assumed seroprevalence values (33%) due to the absence of local seroprevalence data. Future work should incorporate locally measured seroprevalence to improve threshold accuracy and examine the long-term success of these targeted actions and measure their impact on reducing dengue cases. Studies could also explore the role of seasonal changes, community participation, and environmental factors in shaping pupal productivity.

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The authors declared no conflict of interest.

Data Availability Statement
The findings presented in this study are part of a PhD project and draw on data currently documented in the thesis of Nur Aida Hashim.

Contribution of Authors
Nur Aida Hashim contributed to data collection, analysis and manuscript writing. Abu Hassan Ahmad contributed to data interpretation, manuscript final reading and approval.

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