Operational guide for assessing the productivity of *Aedes aegypti* breeding sites

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Operational guide for assessing the productivity of *Aedes aegypti* breeding sites

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Preface

Dengue fever has become a major public health concern in recent decades. The following document is an operational guide on how to perform *Aedes aegypi*[1] pupal productivity surveys. These surveys are used to identify the most productive dengue vector breeding sites so that they can be targeted for interventions.

The use of pupal productivity surveys and the targeted control of the most productive breeding sites, (i.e. those that produce > 70% of all *Ae. aegypti* pupae; with pupae serving as a proxy measure for adult mosquitoes), has been promoted by the World Health Organization (WHO) and the Special Programme for Research and Training in Tropical Diseases (TDR), over the last decade. This strategy is based on the outcomes of multicentre studies of pupal survey techniques and on the cost-effectiveness of targeted interventions (e.g. Focks, 2003; Focks and Alexander, 2006; McCall and Kittayapong, 2007; McCall, Lloyd & Nathan, 2009; Tun Lin et al., 2009; WHO, 2009).

Since the *Ae. aegypti* eradication campaigns of the 1940s in the Americas (Nathan, Focks & Kroeger, 2006), vector infestation levels have been determined by house-to-house surveys investigating the presence of immature stages of the vector (larvae and pupae) in water containers. The results are used to calculate the conventional *Stegomyia* indices: the house (or premise) index, the container index and the Breteau index (the number of positive containers per 100 houses inspected). However, the limitations of these indices in accurately estimating vector densities and ultimately dengue transmission risk, were acknowledged in an informal consultation at the World Health Organization in 1999 (WHO, 2000).

After a decade of revising dengue vector entomological survey methods and indicators (Focks, 2003), a series of studies were conducted under the sponsorship and coordination of TDR and the WHO Department for Neglected Tropical Diseases (NTD). Subsequently, recommendations were issued to incorporate pupal productivity surveys alongside traditional larval surveys to determine the most productive water container types, in order to design more targeted and cost-effective vector-control interventions. The methodology was validated in a nine-country study in Asia, Africa and Latin America e.g. Focks & Alexander 2006; Lenhart et al., 2006), and the cost-effectiveness of targeted interventions by another multi-centre study involving eight countries in Asia and Latin America (Tun Lin et al., 2009). Other studies have followed giving further confirmation to the value of pupal productivity surveys (e.g. Arunachalam et al., 2010; Pilger et al., 2011; Seng et al., 2009).

The following document summarizes the Standard Operational Procedures (SOPs) for conducting pupal productivity surveys.

[1]Although *Aedes albopictus* is found in various countries in Asia and the Americas, *Aedes aegypti* remains the principal dengue vector. This methodology is applicable for the surveillance of both species.

Objectives

This document provides technical guidelines for identifying the breeding sites that are the most important to adult *Ae. aegypti* production, and is intended for field and laboratory personnel working in dengue vector surveillance and control. The relative importance of breeding sites is determined by the proportion of pupae they produce in a given place at a given time; this can serve as a proxy for estimating adult mosquito production. Interventions can then be targeted to control breeding in the most productive containers.

The underlying rationale to this methodology is that counting the number of pupae in breeding sites allows us to identify categories of containers that are producing the most adults. This in turn, can lead to focused vector control activities targeting those containers of greatest epidemiological importance, particularly in high dengue transmission risk areas.

How to define the most productive containers

In ecological terms, 'production' is the abundance of organisms existing in a given place at a given time, and this can be expressed in terms of density (number of individuals per unit of area, volume or other relevant measure).

Ae. aegypti breeding sites are defined as any water-holding containers in which immature stages of *Ae. aegypti* are found. A container is considered 'positive' for *Ae. aegypti* when one or more larvae or pupae are present. However, the simple fact that a container is 'positive' does not offer a measure of its relative importance as a breeding site because it does not provide information on how many individuals develop and are ultimately produced in it.

Counting the number of pupae in each breeding site (to measure pupal productivity) offers insight not only into the abundance of pupae in the container but also an estimate of how many adult mosquitoes may emerge (due to low pupal mortality and the proximity of the pupal stage to the adult stage). Thus, we can assess the importance of breeding sites, establish risk thresholds and focus control operations toward the most productive containers to have the greatest impact on the adult *Ae. aegypti* mosquito populations.

In addition to the number of pupae per container, the number of pupae per person or pupae per hectare can be calculated (Focks, 2003). The associations between these indices and dengue transmission and climate are currently being modelled. In the future such models will help establish thresholds to determine the reductions needed in the vector population to have an impact on dengue transmission at a local level.

To identify the key *Ae. aegypti* breeding sites the percentage contribution of each breeding site to the total count of pupae is calculated. This is done by taking the total number of pupae found in a given category of container and dividing it by the total number of pupae in all containers in the area being studied (Table 1).

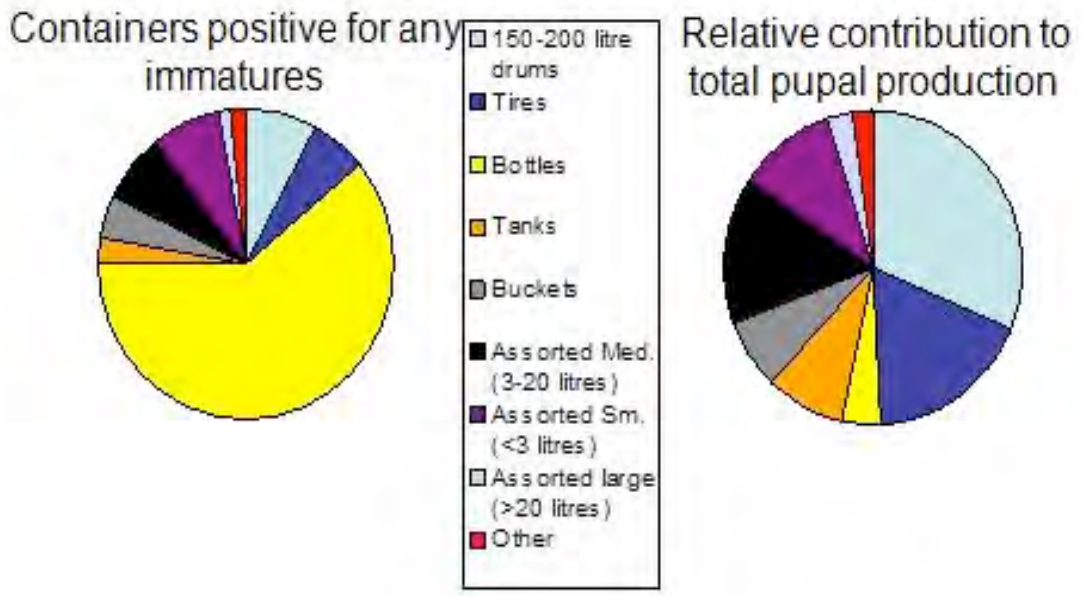
Table 1. How to calculate the relative importance of each type of breeding site: example from Mexico[a].

Container	Total number of containers	Total number of pupae in each container category	Contribution to the total number of *Ae aegypti* pupae (%)
Buckets	2729	279	**2.3**
200 litre drums	724	143	**1.2**
Plastic containers	1393	423	**3.5**
Glass containers	394	149	**1.2**
Ground cement tanks	4082	10 257	**83.6**
Plant pots	521	168	**1.4**
Tyres	230	145	**1.2**
Others	183	102	**0.8**

[a]Adapted from Arredondo-Jiménez & Valdez-Delegado, 2006.

When examining the relative importance of containers, it becomes apparent that the information collected in a pupal survey differs from the information collected in a traditional larval survey. Figure 1 shows results from an entomological survey conducted in the Bolivarian Republic of Venezuela. While bottles are most frequently positive for immatures, they only produce a small proportion of the pupae; conversely, drums and tyres together produce almost 50% of all the pupae, demonstrating that they should be given higher importance for control interventions.

Fig. 1. Containers most frequently positive for immatures (larvae and pupae) are not necessarily those of greatest importance to pupal production.[a]



[a] Adapted from Lenhart et al., 2006.

The equipment and material required to carry out a pupal survey are listed below and illustrated in Figure 2.

- Backpack or field bag
- Clipboard
- Forms
- Labels
- Map of the study site
- Pencil (Black)
- Pencils (Red and blue)
- Permanent marker
- Adhesive tape
- Sieve with extendable handle
- Entomological net (20 cm diameter and 30 cm deep)
- Sieves of different sizes
- White bowl
- 500 ml washing bottle
- Water submersible torch
- 50 ml pipette
- 3 ml pipette
- Vials with caps
- 250 ml cup with a mesh cap

Fig. 2. Field equipment and material required for a pupal survey.

Study design

Pupal productivity surveys should be representative of the area of interest and include households as well as non-residential private and public spaces.

The most suitable study design will be dictated by the particular circumstances of the study site, but will normally consist of a simple random sampling of premises or a cluster design. The latter is a multilevel design where an initial group of neighbourhoods or areas in a city are selected as clusters and either all, or a random sub-sample of premises within them, are surveyed.

A survey of all premises within a cluster is recommended for baseline studies (to obtain initial information), during outbreaks and also for rapid entomological surveillance. The selection of clusters can be done randomly and/or by stratification.

The study design should be determined in advance when pupal productivity surveys are going to be performed within an epidemiologically high-risk area for dengue. The seasonality and frequency of the surveys will depend on the natural fluctuations of the local mosquito populations and disease epidemiology, but at the very least a cross-sectional survey performed during the most intense transmission period is recommended. This is usually the rainy period.

Since premises without immature *Aedes* do not yield information about key and productive containers, sample size calculations only take positive premises into consideration. An algorithm to calculate the sample size is described in Annex I, based on the assumption of a simple random sampling design.

For a cluster sampling design, the algorithm is modified depending on the number of clusters. A survey with a few large clusters will have a lesser degree of precision than one with many small clusters. Thresholds (10, 25, 50, 100) should be multiplied by the *design effect* $D=1+(m-1)\rho$, where m is the expected average of positive premises by cluster and ρ is the correlation coefficient between clusters (between 0 and 1). The *design effect* will be lesser in a survey with small clusters within an area with little variation between clusters in terms of container types, than in a survey with large clusters and a high variation of container types.

Entomological survey

Entomological surveys encompass the active detection of the immature forms (larvae and pupae) of mosquitoes in the selected households/sites.

The surveys are normally conducted by a team of two people, usually entomologists or field technicians with a proper entomological training. Each team is typically able to complete 20 households/sites per day.

At each collection household/site the team must complete the following:
a) A written survey form (Annex II).
b) A careful inspection of the intra- and peri-domestic area looking for water containers with immature forms (larvae and pupae) inside. Only containers holding water are included in the count.
c) Collection of the immature forms (larvae and pupae).

A map of the study area is indispensable. Each household/site includes the sidewalk in front of the household/site, the front garden, the interior part of the house (kitchen, individual rooms, bathrooms etc.) and the backyard. At the end of the survey, it is advisable to put a red dot on the map if the household/site was positive and a blue dot if it was negative. Each dot should also show the identification code (ID) assigned to the household/site (see first entry at the top of the pupae survey form, Annex II).

All constructions and all containers holding water within the site must be checked. Follow a clockwise route when inspecting the peri-domestic area, leaving the centre of the area until the end. Ensure that the whole area is examined.

The survey form

Only containers with water are registered in the survey forms (see Annex II for household surveys and surveys in public spaces).

Each container is classified by its location (see column D of forms in Annex II):

- **Exterior** (any part of the house not covered by a roof, also known as peri-domestic);
- **Interior** (any part of the house covered by a roof, also known as intra-domestic).

Categories of mosquito breeding sites

i) **Ground tanks** – usually made of concrete with an approximate capacity of 1000 litres of water.
Barrels – either plastic or metallic with a capacity between 100–200 litres.
Elevated tanks – usually made of plastic or concrete with a lid and a capacity between 500–1000 litres.


Drum and elevated tank


Barrels


Tank

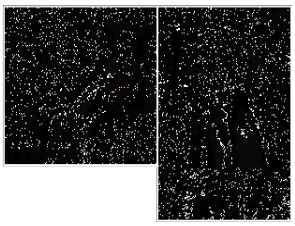
ii) **Cisterns** – usually large (5000–10 000 litres), made of concrete and very often built underground, although sometimes they are on the surface.
Pots and buckets – a plastic or metal container with a capacity of 18–20 litres.
Tyres.


Cisterns


Pots and buckets


Tyres

iii) **Drinking troughs** – water containers to feed animals made of plastic, metal, concrete etc., with different water storage capacities.
Flowerpots – containers for flowers and plants made of clay, metal, ceramic material, etc.
Kitchen/laundry containers – various containers used in the kitchen (pots, pans) and for washing (vats, tubs).


Drinking troughs


Flowerpots


Kitchen/laundry containers

iv) **Broken domestic appliances** – refrigerators, stoves, washing machines, etc.
Bathroom appliances – toilets and wash basins.
Water fountains – usually made of cement, used mainly for ornamental purposes.


Broken domestic appliances


Broken appliances


Water fountains

v) **Assorted small** – all those water containers not mentioned above with a capacity < 5 litres.
Assorted medium – all those water containers not mentioned above with a capacity between 5 and 20 litres.
Assorted large – all those water containers not mentioned above with a capacity above 20 litres.
Bottles.


Assorted small


Assorted medium


Bottles

vi) **Cans**
Others – any other container not listed above.


Cans


Others

Pupae collection

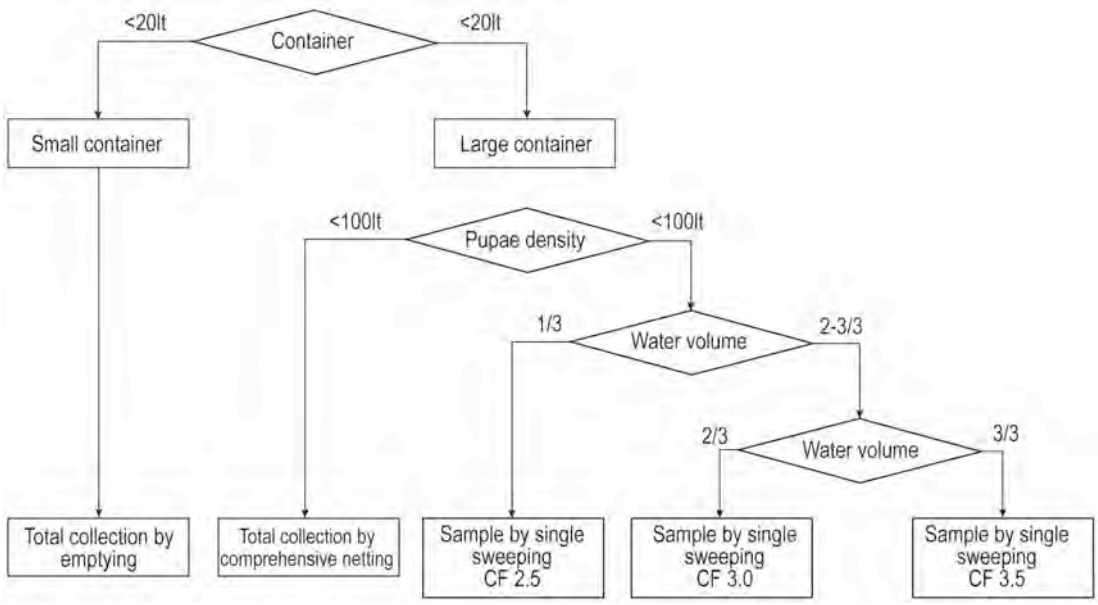
The following sampling strategy (Figure 3) is suggested based on different water container volumes:

If the container has < 20 litres of water, collect all pupae by either emptying the container and sieving the water directly through a mesh net or colander or collecting the pupae with a pipette.

If the container has > 20 litres of water and emptying is not feasible (due to the size or nature of the container) but there is good visibility/low pupae density (< 100 individuals), collect all pupae by comprehensive netting.

If the container is large and has high pupal density (> 100 individuals), collect a sample by sweeping the surface with a net. The total number of pupae can be estimated from this sample by using a calibration factor (CF) according to the amount of water in the container.

Fig. 3. Flowchart illustrating the pupal collection strategy according to container volume.



CF, calibration factor or correction factor.

A sample of larvae can be preserved in alcohol and collected in positive containers to determine the species. All collected pupae should be kept in bags, vials or emerging chambers (for each independent breeding-site) and transported to the laboratory.

Each sample should be labelled with the pertinent information (Figure 4).

Fig. 4. Key information to be included with each sample.

Locality/Municipality:_____

Cluster/Neighbourhood:_____

Premise ID:_____ Date:_____

Container type:_____ Use:_____

Material_____ Collector:_____

Collection from small containers

Pupae from bottles, flasks, tins and other small containers can be emptied directly into a tray or filtered through a sieve, removed with a pipette and stored in vials then transported to the laboratory (Figure 5).

A sieve can be used for capturing pupae from containers that are hard to drain or manipulate, such as car tyres or small fixed containers. Water containing pupae can also be removed from these containers with a large pipette (50 ml) and then sieved.

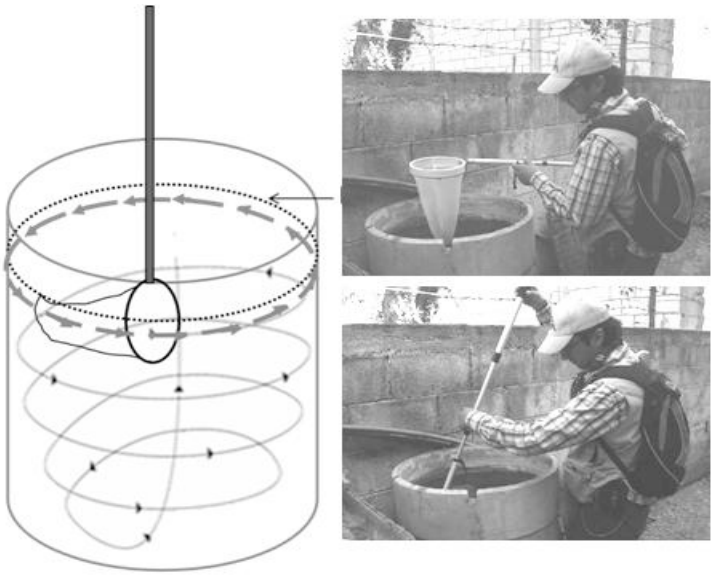
Fig. 5. Collection of pupae from containers that are easy to empty and sieve[a]

[a] Pupae can be removed with ease if placed in a tray of clean water.

Collection from large containers

Collection of pupae by comprehensive netting can be performed in large, round containers with low densities of pupae (Figure 6). The net is immersed carefully 7.5 cm beneath the water surface of the container and moved around the perimeter in a downwards spiral, creating a funnel which concentrates the pupae at the bottom centre of the container. The pupae are then scooped up in the net (modified from Tun-Lin, Kay & Burkot, 1994), collected and then preserved as already described.

Fig. 6. Technique for comprehensive netting



It is necessary to estimate the number of pupae in large containers with high pupal densities (> 100), where an exhaustive and total collection of pupae is impractical (Knox et al. 2007, Romero-Vivas, Llinás & Falconar, 2007). For instance the total number of pupae in a cement water tank can be extrapolated from the number of pupae collected by a single sample, taken from the surface of the container using a net, and then multiplied by one of three correction factors (also called "calibration factors") based on the volume of water present in the container: 1/3 full; 2/3 full or completely full.

Calibration or correction factor (CF): The number of *Ae. aegypti* pupae collected is multiplied by a CF (1/3 CF = 2.5; 2/3 CF = 3.0; 3/3 CF = 3.5). Typical CFs are presented in Figure 3 but if possible the specific CFs should be determined for the important containers at each location.

Laboratory work

All pupae collected from each container are counted and identified by genus to distinguish between *Aedes* spp and *Culex* spp (Annex III) and others.

Only 5–10% of the pupae collected need to be identified to species to confirm that they are *Ae. aegypti* (Annex III).

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ANNEX I

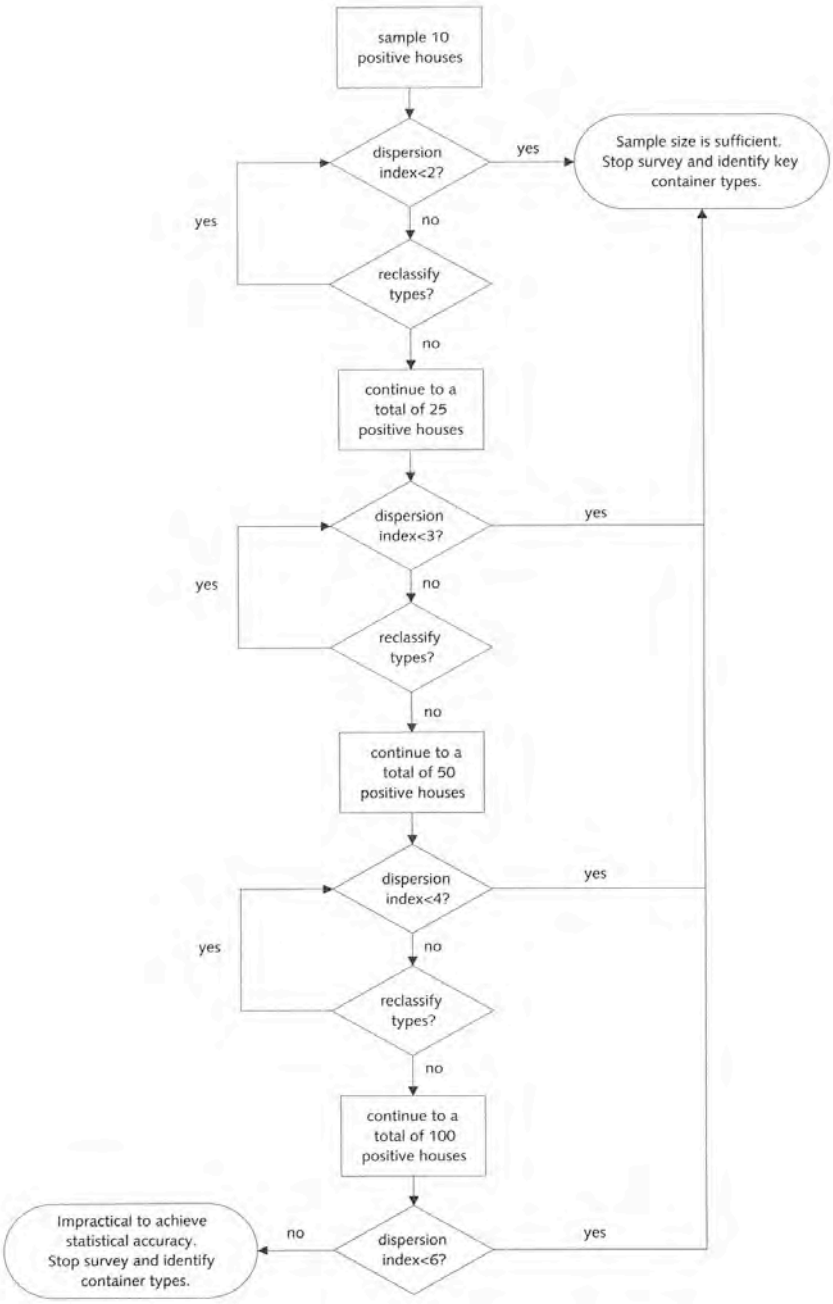
Sample size calculations

The algorithm for calculating sample size is shown in Figure 7 (Focks & Alexander 2006).

Possible sample sizes (10, 25, 50 or 100) are presented in terms of numbers of positive houses. This means that the total number of houses sampled will depend on the proportion that are positive for *Aedes* pupae.

Calculation of the dispersion index (N_1) is shown in Table 2. (The dispersion index is the exponential of H′, the Shannon-Wiener index).

Fig. 7. Flowchart for determining sample size[a]



[a] From Focks & Alexander, 2006.

At each stage if the algorithm indicates an increase in sample size, it is possible instead to recategorize the containers to give fewer types (Table 2), and then to re-analyse the data based on the reduced number of types. In other words, with a smaller number of types, the dispersion index may be small enough to allow the survey to stop. In this case, the key container types should be identified on the basis of the simplified classification.

The sample size should be increased if a cluster survey is being done.

Table 2. Examples of calculation of the dispersion index[a]

(a)	Container type	Proportion of pupae (p)	$\log_{10}(p)$	px $\log_{10}(p)$
	Bucket	0.04	-1.43	-0.05
	Assorted medium	0.07	-1.17	-0.08
	Assorted small	0.01	-1.86	-0.03
	Drum	0.55	-0.26	-0.14
	Tank	0.31	-0.5	-0.16
	Tyre	′0.01	-1.89	-0.02
			H′ = -total =	0.48
			$N_1 = 10^{H'}$ =	3.0

(b)	Container type	Proportion of pupae (p)	$\log_{10}(p)$	px $\log_{10}(p)$
	Bucket	0.04	-1.43	-0.05
	Assorted	0.08	-1.10	-0.08
	Drum	0.55	-0.26	-0.14
	Tank	0.31	-0.5	-0.16
	Tyre	0.01	-1.89	-0.02
			H′ = -total =	0.46
			$N_1 = 10^{H'}$ =	2.9

H′ = Shannon-Wiener Index; N_1 = dispersion index (see page 14)
Note: Table (a) shows the calculation of the dispersion index (N_1) with the original classification of container types and Table (b) merges the two assorted types and reduces the dispersion index. In this case the reduction is small (from 3.0 to 2.9) because one of the original types had few pupae.
[a] Focks & Alexander, 2006

ANNEX II

Instructions for filling out the survey forms

For the surveyor:

- Put tick marks **(√)** in columns 1 to 19 for each container encountered with water.
- Complete columns B to M. Enter the information on water volume in column A only if you are interested in this analysis.

The supervisor will check the form.

The data entry personnel may only use the right half of the form for each container. In this case the supervisor or data entry person has to fill the column container "category" according to the information in column 1 to 19.

ID: [ ][ ][ ][ ][ ][ ][ ][ ]

Entomological Form 1: For Household

Larval/Pupal Survey Form for Households

Surveyed date: dd [ ][ ] — mm [ ][ ] — yy [ ][ ]

Inspector:… … … … …. ….. … … …… … … … … …

Country code: [ ] District/zone: [ ][ ] Cluster: [ ][ ] Household ID: [ ][ ][ ]

If not available specify… … … … …. … … … … … … … … … … … … … .

Number of people living in the house: [ ][ ]

	INDEX	Category A: Water storage containers that are used						Category B: Containers with water that are not used or that are discarded										Category C: Other country specific containers		
		Drum/Barrel	Cement/Steel Tank	Ceramic/Earthern/Fiber Jar	Bucket	Other:(Specify)……………	Other:(Specify)……………	Ceramic Jar	Bowl (Toilet use, fish bowl, ant trap, pet dish)	Flower Vase	Tyre	Coconut Shell	Discarded tins, bottles, plastic containers, broken jars	Natural breeding habitat e.g. plant axil	Other country specific (Specify)…………	Other country specific (Specify)…………	Other country specific (Specify)…………	Other(Specify)……………	Other(Specify)……………	Other(Specify)……………
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Each water container has one row to be filled (containers with water only)	1																			
	2																			
	3																			
	4																			
	5																			
	6																			
	7																			
	8																			
	9																			
	10																			
	11																			
	12																			
	13																			
	14																			
	15																			
TOTAL																				

	Details of each container	INDEX	CATEGORY	Water volume (capacity volume)	Type of water (1=tap/well, 2=rain, 3=other)	Under vegetation (Fully=1;Partially=2; Not at all=3)	Container Location (I=Inside; O=Outside)	Usage during the past 7 days (1=yes, 2=no)	Shade:1 =fully; 2 =partially; 3 =nil	Intervention applied to this container? 1=Yes; 2=No	Cover (Proper=1;Partial=2;No=3)	*Larval density: 0=no larvae,1=<10; 2=10-50; 3 =>50	*Pupae count: Absolute number (small containers)	*Pupae count: Sample / correction factor	*Pupae count: Estimated Pupal count (large container)	Remarks
				A	B	C	D	E	F	G	H	I	J	K	L	M
Each water container has one row to be filled (containers with water only)		1														
		2														
		3														
		4														
		5														
		6														
		7														
		8														
		9														
		10														
		11														
		12														
		13														
		14														
		15														
TOTAL																

NOTE: CATEGORY COLUMN SHOULD BE FILLED IN BY FIELD SUPERVISOR. FOR FIELD SUPERVISOR, PLEASE USE THE NUMERIC CODE FROM EACH CONTAINER CATEGORY AS STATED IN THEIR COLUMN'S HEADING AND FILL IN THE CODE IN THE CATEGORY COLUMN.

ID

Entomological Form 2: For Public spaces

Larval/Pupal Survey Form for Public spaces

Surveyed date: dd __ mm __ yy

Inspector:

Country code: District/zone: Cluster: Type of public space (code below)

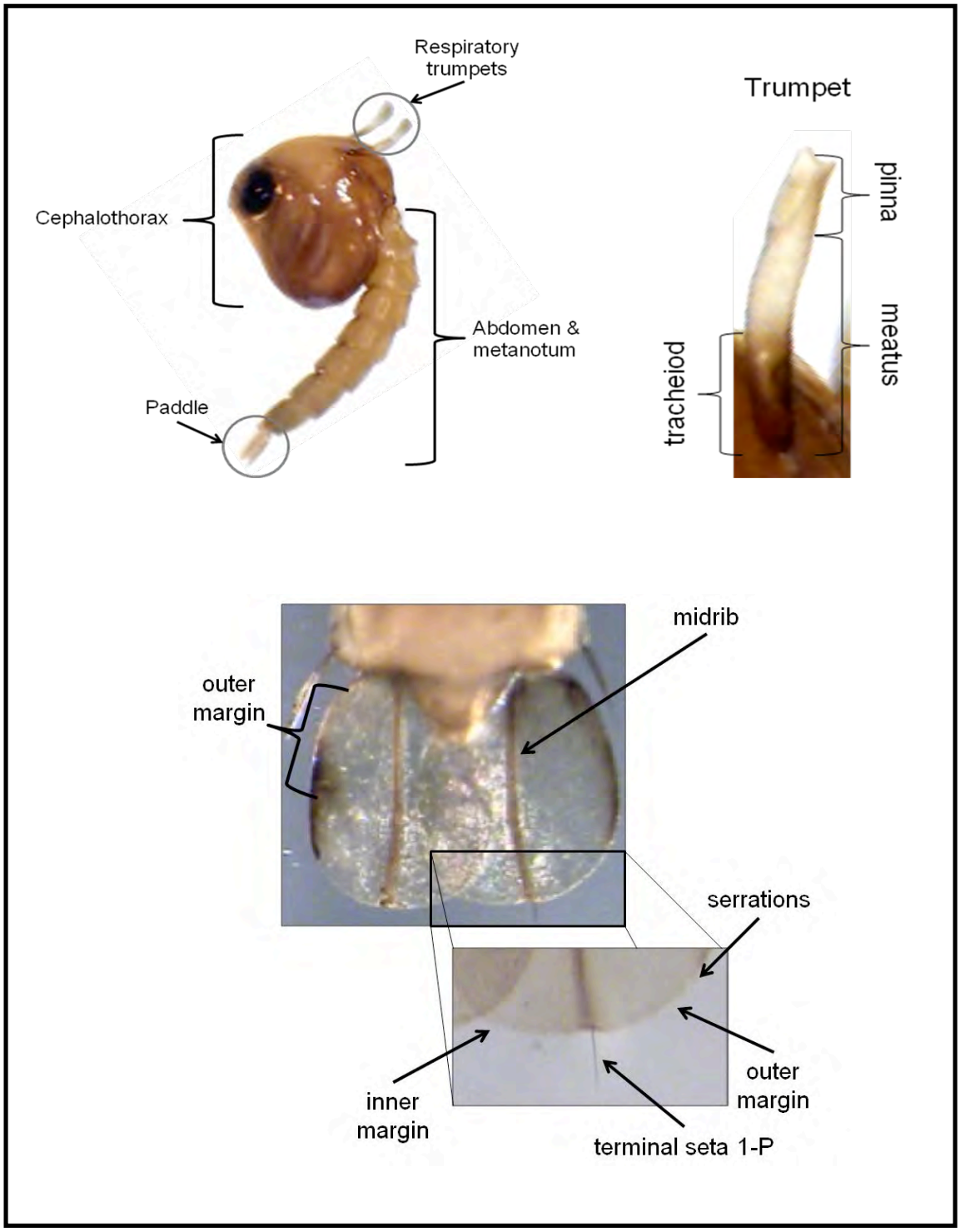
1= public street/pathway; 2= Green area for leisurel; 3. Abandoned area, dumping ground; 4= Public building (school, hospital including garden/open space; 5= Religius building including open space/garden; 6= Private business (if not included in HH survey)

		Category A: Water Storage Containers that are used						Category B: Containers with Water that are not used or that are discarded									Category C: Country Specific Containers			Details of each container																
	INDEX	Drum/Barrel	Cement / steel Tank	Ceramic / Earthern / Fiber Jar	Bucket	Other:(Specify).....	Other:(Specify).....	Ceramic Jar	Bowl (Toilet use, fish bowl, ant trap, pet dish)	Flower Vase	Tyre	Coconut Shell	Discarded tins, bottles, plastic container, broken jar	NUN:Natural breeding habitat e.g. plant axil	Other country specific (Specify).....	Other country specific (Specify).....	Other country specific (Specify).....	Other(Specify).....	Other(Specify).....	Other(Specify).....		INDEX	CATEGORY	Water volume(capacity volume)	Type of water (1=tap/well, 2=rain, 3=other)	Under Vegetation (1=Fully; 2=Partially; 3=Not)	Container Location (I=Inside, O=Outside)	Usage the past 7 days)1=yes; 2=no)	Shaded: 1=fully; 2=partially; 3=not	Intervention applied to this container? (1=Yes, 2=No	Cover (Proper=1;Partial=2;No=3)	*Larval density	*Pupae count			Remarks
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19				A	B	C	D	E	F	G	H	I	J	K	L	M
Each water container has one r ow to be filled (containers with water only)	1																					1													
	2																					2													
	3																					3													
	4																					4													
	5																					5													
	6																					6													
	7																					7													
	8																					8													
	9																					9													
	10																					10													
	11																					11													
	12																					12													
	13																					13													
	14																					14													
	15																					15													
TOTAL																																			

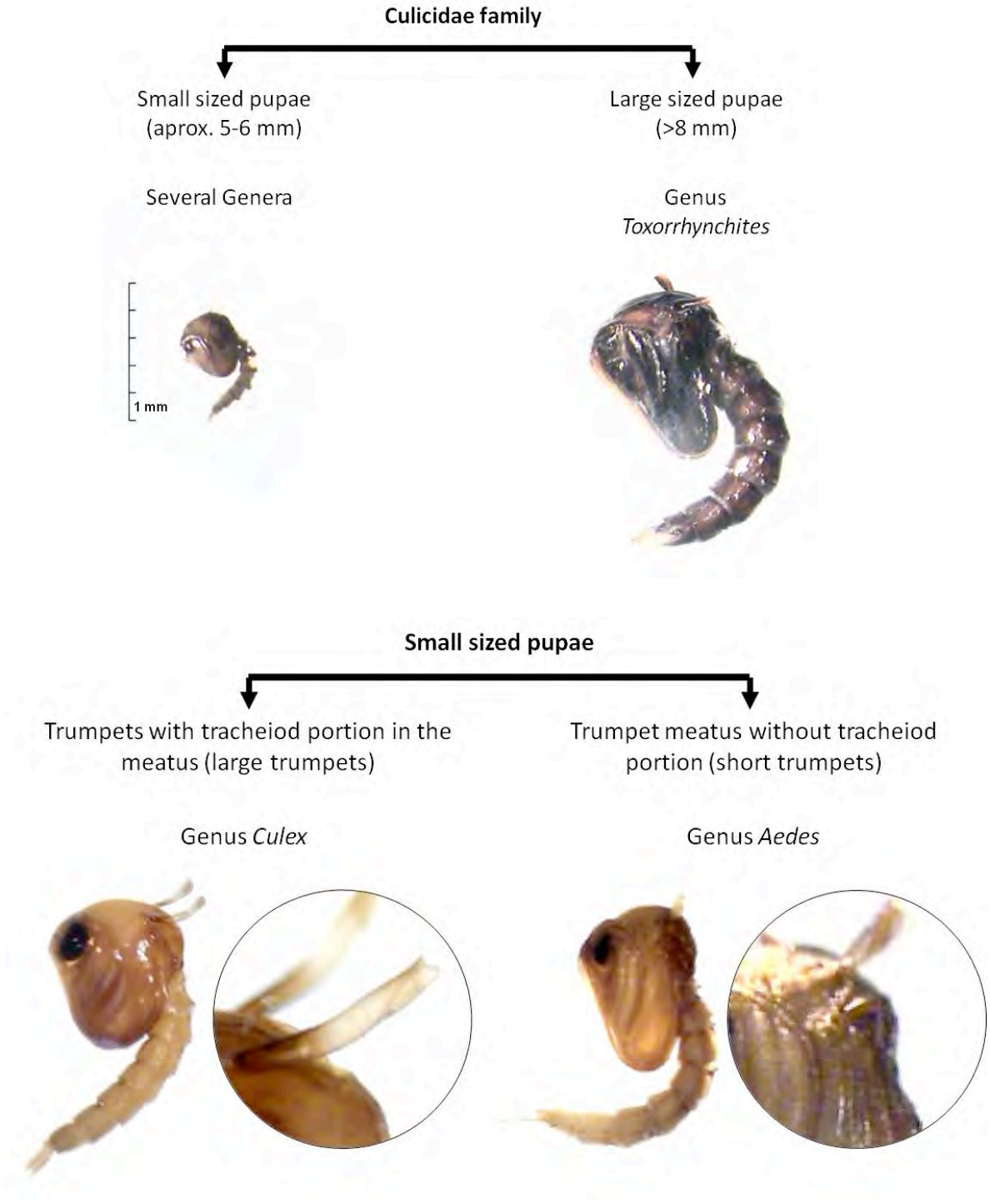
NOTE: CATEGORY COLUMN SHOULD BE FILLED IN BY FIELD SUPERVISOR. FOR FIELD SUPERVISOR, PLEASE USE THE NUMERIC CODE FROM EACH CONTAINER CATEGORY AS STATED IN THEIR COLUMN'S HEADING AND FILL IN THE CODE IN THE CATEGORY COLUMN.

ANNEX III

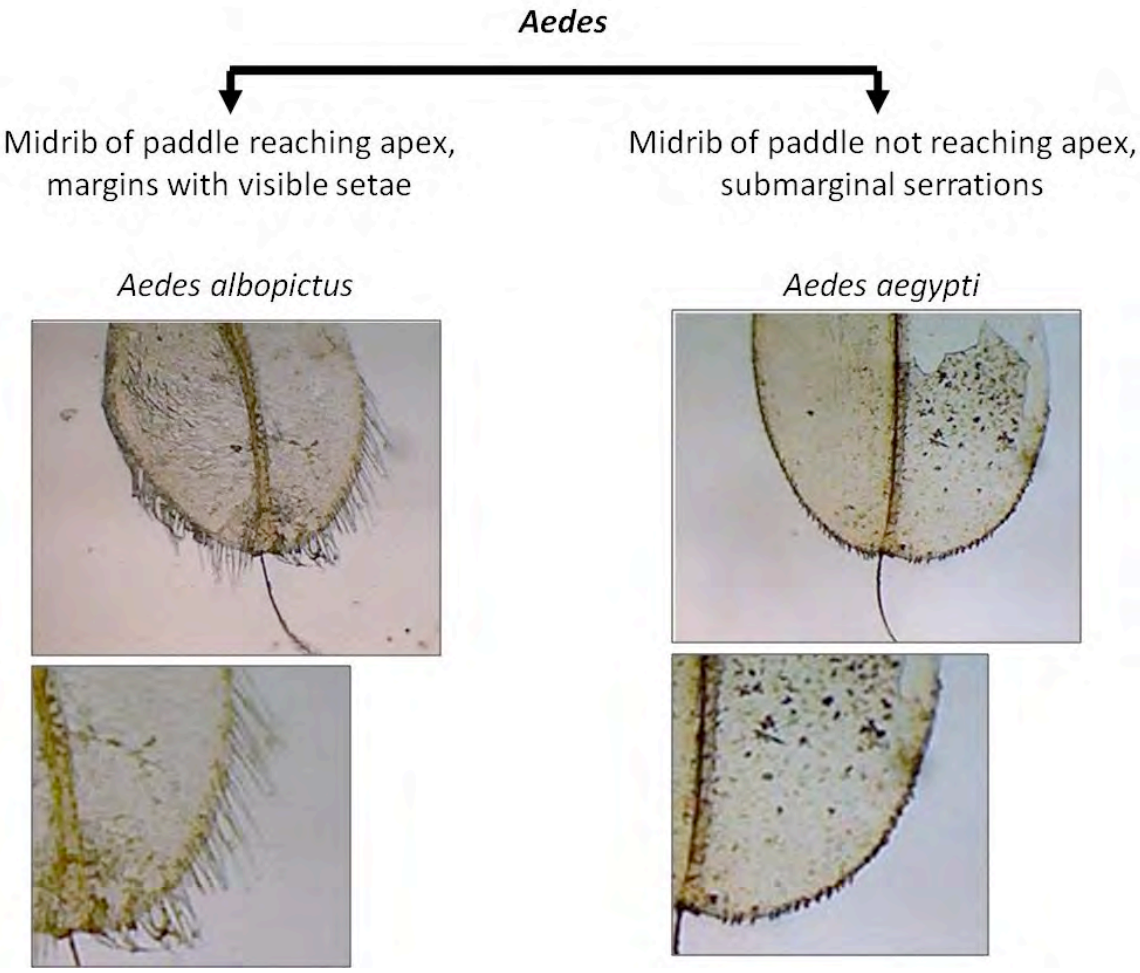
Photographic key for pupae



Photographs: Cassandra González-Acosta & Azael Che-Mendoza.



Photographs: Cassandra González-Acosta & Azael Che-Mendoza.



Photographs: Cassandra González-Acosta & Azael Che-Mendoza.

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