Standard Operating Procedure for conducting larval and pupal surveys for *Aedes*

Effective Date: 20 June 2022

SOP #: LAE-2021

PacMOSSI
Pacific Mosquito Surveillance Strengthening for Impact

Scope

This Standard Operating Procedure (SOP) describes the materials and methods to perform surveys of immature mosquitoes in container habitats (generally *Aedes* species). This SOP uses information from documents referenced.

Overview

Description: Larval sampling involves capturing immature mosquitoes from aquatic habitats.

Target species and physiological states: Captures larvae and/or pupae of many species.

Entomological surveillance indicators: Larval sampling is used for the full range of immature vector indices, and in addition, larvae can be reared and used as specimens for insecticide resistance bioassays.

Advantage: The equipment and supplies and inexpensive and portable.

Disadvantage: Larval sampling is a poor indicator of adult production. Larval surveys are labour intensive and require well trained staff. Experience is important as the more sampling a person does, the better at larval surveys one becomes. Cryptic habitats are easily missed. It can be difficult identify larvae to the species level.

Data: Total number of larvae and pupae per aquatic habitat or dipping effort. Larvae can be recorded by early or late instars. Alternatively, presence or absence at each site can be recorded. When necessary, field data is merged with the results of subsequent laboratory analyses.

Materials

- ◯ 3 ml plastic pipette with end cut off
- ◯ 50 ml pipette/irrigation syringe
- ◯ Aquarium fish net
- ◯ White plastic tray
- ◯ Larval collection vials
- ◯ Pen/pencil
- ◯ Data collection form/digital device
- ◯ Ethanol (95 – 100%)
- ◯ Cooler box (optional)

Description of *Aedes* larval habitats

The potential vectors of dengue, Zika and chikungunya in the Pacific region are *Aedes aegypti, Aedes albopictus, Aedes polynesiensis, Aedes cooki, Aedes hensilli, Aedes pseudoscutellaris, Aedes rotumae, Aedes scutellaris* and *Aedes hebrideus*.

The larvae of these species can be found a variety of natural and human-made freshwater habitats, and their preference to use different aquatic habitats varies by species. Natural aquatic habitats (more commonly used by *Ae. albopictus* and *Ae. polynesiensis*) include: tree holes, leaf axils, broken coconuts and shells. Artificial sites (often referred to as containers and commonly used by *Ae. aegypti*) include: water tanks or storage vessels, car tires, discarded plastic, metal and glass containers, buckets, pot plant bases or water trapped in tarpaulins. These species may also utilise hard to find habitats such as gutters, drainage pipes or subterranean pits which hold water.

Containers that are highly nutrient rich, such as sewerage pipes, may be more attractive to certain *Culex* species (which may transmit Ross River virus or lymphatic filariasis).

Assessing the availability and occupancy of aquatic habitats requires systematic searches for potential habitats throughout the entire village area, including inside the boundaries of all properties.

Figure 1. Potential larval habitats for *Aedes*. A) Shell, B) car tires, C) discarded styrofoam, D) Broken boat, E) Coconut & F) Discarded plastic bottle.
Photos from Dr Kyran Staunton.

Sampling procedure

Systematically search each premise for potential aquatic habitats. Immature surveys should favour areas near human habitation and high risk for *Aedes*-borne viruses, and any habitat of any size that holds water should be carefully inspected.

1. **Gather all equipment required.**

2. **Many containers are associated with households and surrounding areas so first permission to work in a house hold must be obtained. Once consent by the home-owner has been given to inspect their premises, move around the area looking for larval habitats, both inside and outside of the house.**

3. **The sampling method may need to be adjusted depending on the size of the container.**

   a. **Large containers (e.g. 200 L drum or rainwater tank) can be surveyed using an aquarium net or a dipper.**

      - **For sweep nets, a standardised five-sweep method can be used for medium-to-large sized larval containers (Knox et al. 2007)**

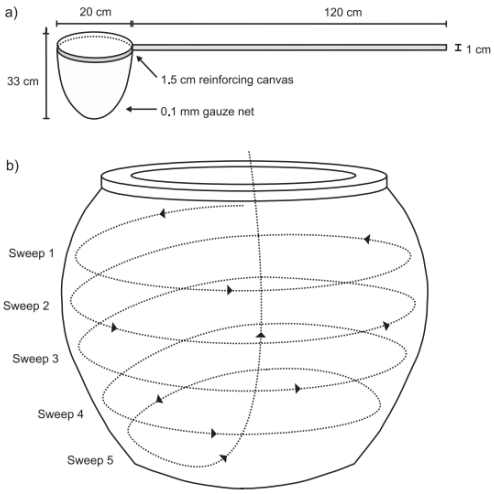
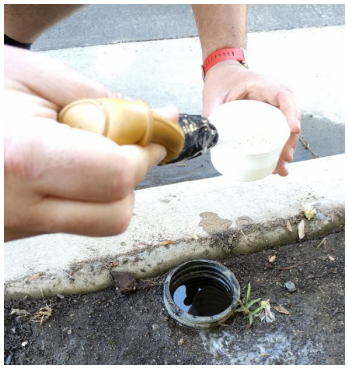
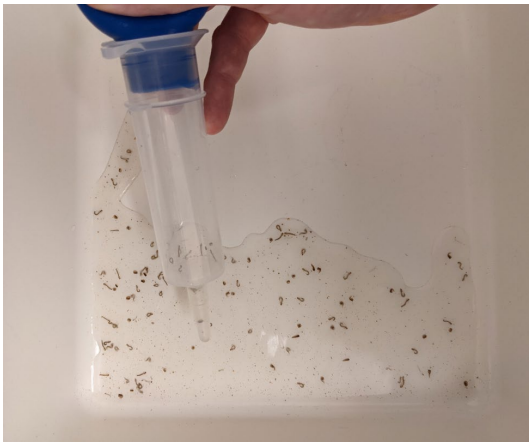


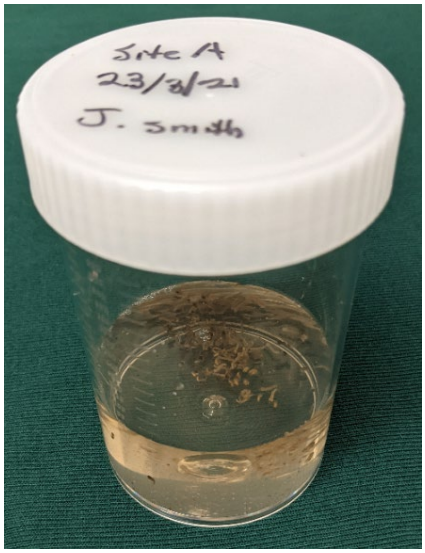
Image: Knox et al. J Med Ent.

   b. **For small containers a pipette can be used to directly remove larvae or the entire contents can be tipped onto a white tray and the larvae picked out.**



c. For discarded car tyres, use a small aquarium net or a 30 ml pipette.	
d. For tree holes and leaf axils, pipette out the contents with a 30 ml pipette. You may need to add water to the holes to flush out larvae in the debris at the bottom.	
e. For crab holes, pump out with a pump and a long hose attached.	
4. Transfer the collected samples using a pipette into a vial.	

5. **Seal and label the vial containing the immature mosquitoes.**
    a. Ensure that not too many larvae are placed in the vials (approximately 2 ml of liquid for each larvae). A 50 ml vial may have 40 ml of water and 20 larvae in it.



6. **To transport larvae, they can be stored in some of the water from the habitat. During transport keep them cool and consider using a cooler box to protect them.**

7. **Larvae can be killed with hot water from a kettle or by putting into a vial with >70% ethanol. The samples of larvae and pupae can be stored in 70% ethanol for an extended period. Be sure not to dilute the ethanol below 70% as the mosquitoes may decompose and not be identifiable or useful for further analyses.**

Additional notes:

- Take the GPS coordinates with the tablet provided or record the location data on a map or by household identifier.

- To identify key larval habitats, the percentage contribution of each habitat type to the total count of pupae is calculated (the total number of pupae in a container type is divided by the total number of pupae collected in all containers)

- It's easy to be confident of positive sampling, it's much harder to be confident of negative sampling.

- Newly emerged 1st instar larvae are very hard to see – but might still be there in large numbers.

Videos

The watch a video on how to conduct larval surveillance go to:

- PacMOSSI How to conduct larval and pupal surveys for *Aedes* – https://youtu.be/2WJqju5stAE

Safety/Risk assessment

Your workplace may require you to complete a risk assessment prior to conducting field work. There are a range of risks to which field workers could be exposed, and when conducting larval sampling may include:

- Mosquito transmitted infections
- Dog bites

For further details on safety and risk assessments see SOP# MOS-2021.

References

Knox, T. B., Yen, N. T., Nam, V. S., Gatton, M. L., Kay, B. H., & Ryan, P. A. (2007) 'Critical Evaluation of Quantitative Sampling Methods for *Aedes aegypti* (Diptera: Culicidae) Immatures in Water Storage Containers in Vietnam.' *Journal of Medical Entomology*. https://doi.org/10.1603/0022-2585(2007)44[192:CEOQSM]2.0.CO;2

USAID & ZAP. (2017) 'Entomological surveillance protocol of immature *Aedes aegypti* mosquitoes (larvae/pupae).' http://www.africairs.net/wp-content/uploads/2019/08/Larvae-Pupae-survey-ZAP-protocol_English.pdf

WHO & TDR. (2011) 'Operational guide for assessing the productivity of *Aedes aegypti* breeding sites.' https://www.who.int/tdr/publications/documents/sop-pupal-surveys.pdf

Citation

Tanya L Russell, Kyran Staunton, Thomas Burkot. 2022. 'Standard Operating Procedure for conducting larval and pupal surveys for *Aedes*.' protocols.io