Larval Surveillance Procedures

(Modified from Virginia Arbovirus Surveillance Response Plan, 2004)

Basic tools: Standard, white 400 ml-capacity dipper; an eyedropper; turkey baster, tea strainer, modified bilge pump, white enamel or plastic pan, boots, vials, 6 oz. plastic bags or some other form of container for collecting larvae; labels for the collections; and a pencil. A GPS receiver should also be used to obtain geo-reference [latitude and longitude] data for GIS mapping.

Potential Breeding Habitat: Mosquitoes can breed anywhere there is standing water such as: tires; bird-baths; plant pots; storm drains; and neglected, un-chlorinated swimming pools. Natural breeding habitats include: temporary flooded areas; ditches; tidal or freshwater wetlands; and other areas with temporary, or seasonal standing water. Permanent bodies of water such as lakes or stream pools may also contain the larvae of a few mosquito species in shallow areas, areas of emergent vegetation, or areas with floating debris or vegetation. Flowing water or bodies of water subject to wind or wave action are not suitable breeding habitat for mosquitoes.

When searching for mosquito larvae, it is necessary to proceed slowly and carefully. Approach the area to be inspected with caution, as heavy footfalls will create vibrations that disturb larvae and cause them to dive to the bottom. Likewise, avoid disturbance of the water, as this will have the same result. Approach the area to be sampled with the sun in one's face; this prevents shadows that also disturb larvae and cause them to dive. If wind is of significant magnitude dipping should be done on the windward side of the habitat where larvae and pupae will be most heavily concentrated.

Mosquito larvae are usually confined to the margins of a body of water and will not be found in open, deep water. Dipping should be done around floating debris, aquatic and emergent vegetation, logs and tree stumps in the water, and grasses around the margins. Look for the presence of larvae and pupae before beginning to dip.

One must also recognize that each area to be checked may contain a number of different microhabitats, and each may contain the larvae of different species. Learn to recognize different microhabitats within an area; each one of these should be sampled in order to obtain a comprehensive picture of the area's species composition.

Collection Methods: The kind of mosquito one is looking for, as well as the type of habitat one is working in, will determine the dipping technique used. The following eight techniques for sampling mosquito larvae and pupae with the standard pint dipper are effective:

1. The Shallow Skim - *Anopheles* larvae are normally found at the surface of the water among aquatic vegetation or floating debris. They can be collected with a shallow, skimming stroke along the surface, with one side of the dipper pressed just below the surface. End the stroke just before the dipper is filled, to prevent overflowing.

2. Partial submersion - Around emergent vegetation, logs and tree stumps, larvae may be drawn into the dipper by submerging one edge so that the water flows rapidly into the dipper. In this method, the dipper is stationary.

3. Complete submersion - Certain Culicine larvae (such as species of *Aedes* and *Psorophora*) are very active and usually dive below the surface when disturbed. In this case, a quick plunge of the dipper below the surface of the water is required, bringing the dipper back up through the submerged larvae. Bring the dipper back up carefully, to avoid losing the larvae with overflow.

4. Dipper as a background - This is an especially useful technique in woodland pools, for early season species. Submerge the dipper completely within the woodland pool, going down into the bottom litter if necessary. Use the white dipper as a background against which larvae and pupae can be spotted. Come up underneath the larvae with the dipper. Once again, bring the dipper up carefully, to avoid losing its contents.

5. Flow-in method - This method is useful in situations where the water is shallow, with mud, leaf litter, or other debris on the substrate. Specimens can be collected by pushing the dipper down into the material on the bottom and letting the surface water and mosquito larvae flow directly into the dipper.

6. Scraping - This method is used in permanent or semi-permanent habitats containing clumps of vegetation, such as reeds or tussocks. Dip from the water in, towards the tussock, and end by using the dipper to scrape up against the base of the vegetation to dislodge any larvae present.

7. Simple scoop - This is the technique that seems to be most commonly used by field personnel for larval surveillance and is the one referred to in much of the literature as "the standard dipping procedure." The technique involves simply scooping a dipper full of water out of a habitat. It is useful in a wide variety of habitats, especially for collecting *Culex*.

8. Salt marsh - As the name indicates, this is a procedure to utilize when conducting salt marsh larval surveillance. In the case of salt marsh potholes, dip in a number of spots around the edge of the pothole, dipping in toward the edge. Sample the middle of the pothole, using either a skimming or scooping stroke. In areas containing numerous potholes, make sure several are sampled, not just one or two. Use the same combination of techniques to sample a salt marsh pan.

It is important to recognize that there are different techniques that can be used in different habitat types. Whenever dipping for immature mosquitoes, regardless of the technique used, it is important to look for actual presence of larvae before dipping, and to proceed carefully and pay attention to what you are doing.

Several species of mosquito may be difficult to collect by dipper because their aquatic habitats often occur in containers or other depressions that are too small to sample with a dipper. These include:

- *Ae. albopictus* - tires
- *Ae. atropalpus* - rock pools, tires
- *Ae. triseriatus* - treeholes, tires, containers
- *An. barber* - treeholes, tires, containers
- *Cq. perturbans* - permanent water with emergent vegetation
- *Cs. melanura* – occasionally tires
- *Or. alba* - treeholes, tires, containers
- *Or. signifera*: - treeholes, tires, containers
- *Tx. r. septentrionalis* - treeholes, tires, containers
- *Wy. smithii* - pitcher plants

The turkey baster is an inexpensive, readily available tool that is very useful for sampling tires, containers and tree-holes. A small white plastic soup ladle will also work well. The tea strainer can be used to concentrate and sort samples. Modifying a hand-operated bilge pump by removing the intake valve converts the pump to a syringe capable of drawing up a column of water (Walker and Crans, 1986). The modified bilge pump can also be used to sample treeholes, tires, underground crypts, and various other containers. Material collected by bilge pump or baster can be emptied into a white enamel pan, from which the mosquito larvae are then spotted and removed.

Measurement of Density: Larval density is almost always expressed as numbers of larvae and pupae per dip. Density should be expressed in real numbers. That way, one knows exactly what one is dealing with in terms of population size. Belkin (1954) developed a simple index for determining larval densities that some may prefer to use:

BI = TLP/ND x BP

BI = the breeding index

TLP = the total number of larvae and pupae taken

ND = the number of dips

BP = the number of breeding places

A "breeding place" is defined as each separate microhabitat or station within a site from which one to three positive dips are obtained.

Data Collection: If you are unable to use the computerized database for larval surveillance data, a “Larval Surveillance Data Form” should be completely filled out for each collection. Consult the attached “Code Sheet” for breeding site description and control options. The larval surveillance form may also be a useful means of collecting field data that can subsequently be entered into the computer larval surveillance database.

LARVAL MOSQUITO SURVEILLANCE LOG

Collection Date

Collector

Property Owner
USACE, Norfolk District

Point of Contact (POC)
Keith Lockwood, OPS-TSS

Map Coordinates
Latitude
Longitude

Location
Craney Island Dredged Material Management Area

POC Telephone
(757) 201-7127

Mosquito Aquatic Habitat (circle one)

1. Lake/Pond Margin
2. Permanent Wetland/Marsh
3. Seasonal Wetland/Marsh
4. Saltmarsh/Tidal Pool
5. Vernal Pond
6. Forest Flood Pool
7. Field Flood Pool
8. Puddle
9. Receding River/Creek Pool
10. Rock Pool
11. Sluggish Stream
12. Flooded Ditch
13. Storm-sewer Pool (underground)
14. Storm-water Pond (BMP)
15. Large Artificial Container (>30 gal.)
16. Small Artificial Container
17. Containment Cell (CIDMMA)
18. Other: (specify)

Larvae Present (circle one) **Yes** **No**
Pupae Present (circle one) **Yes** **No**
Eggs Present (circle one) **Yes** **No**

Predominant Instars (circle one)

1. 1st Instars
2. 2nd Instars
3. 3rd Instars
4. 4th Instars
5. Pupae
6. Mixed 1st & 2nd instars
7. Mixed 2nd & 3rd instars
8. Mixed 3rd & 4th instars
9. Mixed 4^{th} instars & Pupae
10. Mixed all stages

Total # of Larvae
Number of Dips

Control Measure Applied (circle) **Yes** **No**

Control Measure Applied (circle one)

1. Dumped/Drained
2. Larvicide (B.t.i.)
3. Larvicide (B.s.)
4. Larvicide (Altosid)
5. Mosquito Fish
6. Herbicide

Notes

Characteristics of Aquatic Habitat (circle one or more)

1. Muddy
2. Clear
3. Salty
4. Fresh
5. Polluted, Organic Waste
6. Polluted, Mineral Waste
7. Polluted Organic/Mineral
8. Floating Debris/Trash
9. Algae Turbid (green water)
10. Algae Crusted (bottom/sides)
11. Algae Floating
12. Aquatic Emergent Grasses
13. Aquatic Floating Vegetation
14. Peat choked (black water)
15. Live Grass Choked
16. Dead Grass Tea
17. Live Vegetation Choked
18. Dead Vegetation Tea
19. Dredged Material
20. Stagnant