Guidelines for Larval Surveillance [1]

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Abstract

Routine surveys for larval mosquitoes should be an ongoing function of every mosquito control agency but the importance of larval surveillance is often overlooked. This paper discusses the advantages of larval surveillance and the methodology for sampling as well as data management. Basic guidelines are presented to help establish the minimum standards required in the development of a larval surveillance program.

Introduction

As early as 1922, members of the New Jersey mosquito control community were engaged in debate concerning the relative merits of "night collections" as opposed to larval collection and identification (Headlee, 1922; Jackson, 1922). Some of the past practices of mosquito control organizations within our own state included treating any standing water encountered, regardless of whether or not mosquito larvae were actually present. Guidelines for starting a mosquito control program included the advice that directors should not spend an excessive amount of time on surveys (Lowe, 1977). Even now, some individuals feel that larval surveys are really only necessary in the early part of the breeding season; once it has been established what particular species are present in each specific site, it can be taken for granted that the species composition will remain the same throughout the rest of the season.

Advantages of Larval Surveillance

These attitudes notwithstanding, surveys of immature mosquitoes are important aspects of an effective mosquito surveillance and control program. They are used to determine the location, species and population densities of pest and vector mosquitoes. They are vital for predicting adult emergence and establishing optimal times for application of larval control measures. They are utilized to forecast the need for adult mosquito control, as well as to assess the effec-

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tiveness of both chemical and biological control measures (Driggers, *et al.*, 1978). The advantages of conducting larval surveillance are:

- Routine larval surveillance provides a more complete and accurate record of sources of mosquito breeding, thereby providing documentation of mosquito production as a basis for treatment.
- Ongoing larval surveillance allows for continuous evaluation of insecticide application and control results.
- A clear understanding of species distribution, density and seasonal occurrence is facilitated through routine larval surveillance.
- Routine larval surveillance enhances the knowledge provided by adult mosquito surveillance (*e.g.*, light traps, bite counts, landing rates).
- A system for the detection of insecticide resistance is provided through a larval surveillance program (Reed and Husbands, 1969).

Throughout this paper, the term "larval surveillance" will refer to the determination, by whatever means, of the presence or absence of immature mosquitoes within a given site. In addition, larval surveillance will also imply to the collection of immature mosquitoes, their identification and some form of recording these data.

Administrators of control programs may feel that the time and effort devoted to this kind of sampling and documentation cannot be justified. These objections may indeed be valid, but there are justifications which are just as valid. In the recently enacted Freshwater Wetlands Protection Act, condition #7 for general permit #15 for freshwater wetlands management projects states: "The Administrator, Office of Mosquito Control Coordination, determines that the project is necessary to abate a documented mosquito problem." The operative - word here is "documented"-the applicant must furnish relevant data indicating that the area to be managed is a site of mosquito production. This cannot be done without performing larval surveillance.

Another justification for the performance of larval surveillance is that surveillance data can be used as an aid in determining those areas within a county's inspection and treatment system which can be eliminated. Areas habitually producing non-vector or non-nuisance mosquitoes can be dropped, thus saving time and money. In consideration of the cost of temporary abatement, the benefits of larval collection and identification are certainly worth the additional effort (Lombardi and Imber, 1976).

A poll of the county mosquito control agencies in New Jersey revealed that the major reasons for carrying out larval surveillance were: 1) to determine the need for temporary control and to direct these control efforts to the appropriate sites, 2) to identify mosquito species occurrence and distribution within the individual counties, and 3) to justify the need for and performance of source reduction work.

The Basics of a Larval Surveillance Program

It is important to organize known breeding sites into some type of catalog or system. This can be in the form of a route book, a map system, or a set of index cards. Whatever form is chosen, a clear system is easier to follow and makes record keeping simpler and more efficient. Areas can be added and deleted as necessary.

The basic tools required for larval surveillance are: a standard, white 400 ml-capacity dipper; a small pipette or eyedropper; a pair of boots, vials, 6 oz plastic bags or some other form of container for collecting larvae; labels for the collections; and a pencil. Other, more specialized tools may be necessary for sampling larval habitats inaccessible to a dipper; this will be covered in more detail later.

Knowledgeable, interested personnel are of paramount importance in a good larval surveillance program and training is a key element in obtaining this goal. Field personnel need to be able to tell the difference between mosquito larvae and pupae and mosquito-like Diptera, such as *Mochlonyx*. They should also be familiar with the major pest and vector mosquitoes encountered in their county, their seasonal occurrence and typical breeding habitat. In-house training is an excellent means of ensuring that one's field personnel are knowledgeable and responsible. This is one of the reasons why it is important for each county mosquito control organization to have a surveillance specialist on staff.

Larval Sampling Techniques

Mosquito larvae are found in a great variety of habitats. This fact has created a need to develop a number of different sampling techniques to ascertain the presence or absence of immature mosquitoes, and to estimate their numbers (Hatfield et al., 1985). The kind of mosquito larvae one is looking for will determine the sampling technique to be used. As mentioned earlier, it is important that field personnel know the preferred breeding habitats and seasonal occurrence of species known or suspected to be present within an area (Sholdt, 1971).

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 which 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 found where surface vegetation or debris are present. In larger pools and ponds, they will usually be confined to the margins 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.

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. If field personnel are familiar with the general breeding habits of the major species found within their county, they will be able to choose the most appropriate technique to obtain the most reliable results. We have formulated the following eight techniques for sampling mosquito larvae and pupae with the standard pint dipper:

- 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.
- 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 within the water.
- Complete submersion—Certain *Culicine* larvae (such as species off *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 current.
- 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.
- 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 shadow surface water and mosquito larvae flow directly into the dipper.
- Scraping—This method is used in permanent or semi-permanent habitats containing clumps of vegetation, such as 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.
- Simple scoop—This is the technique which 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 dipperful of water out of a habitat. It is useful in a wide variety of habitats, especially for collecting *Culex*.

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

Surveillance for Larvae That Are Not Collected by Dipping

Table 1 contains a list of mosquito species found in New Jersey which are not routinely collected with a dipper. *Aedes albopictus* is included, even though it has not yet been found in New Jersey.

Some alternate sampling devices for collecting these species are: suction meat basters, tea strainers, modified bilge pumps and hospital syringes. Starting with the suction meat baster-this is an inexpensive, readily available tool that is very useful for sampling tires, containers and treeholes. One can also use this device for *Cs. melanura* surveys if the collector does not mind reaching down into cedar and red maple root cavities and probably getting dirty in the process. The tea strainer can also be used in *Cs. melanura* surveys. In this case, the collector scoops.

Table 1. Mosquito larvae that are not routinely collected with the dipper

Species	Habitat
Ae. albopictus	Tires
Ae. atropalpus	Rock pools, tires
Ae. triseriatus	Treeholes, tires, containers
An. barberi	Treeholes, tires, containers
Cq. perturbans	Permanent water with emergent vegetation
Cs. melanura	Cedar and red maple swamps, occasionally tires
Or. alba	Treeholes, tires, containers
Or. signifera	Treeholes, tires, containers
Tx. r. septentrionalis	Treeholes, tires, containers
Wy. smithii	Pitcher plants

material out of the root cavity with the strainer and deposits the material in an enamel pan to sort for the larvae. A more convenient tool for collecting this species is the modified boat bilge pump. Removal of the intake valve on a hand-operated bilge pump 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 and various other containers. Again, white enamel pans are a very useful accessory to both the suction meat baster and bilge pump; the material collected with these devices is emptied into the pan, from which the mosquito larvae are then removed.

When sampling pitcher plants for *Wy. smithii*, either a meat baster or a pipette can be used to remove water and larvae from the plant leaves.

Surveillance for *Cq. perturbans* larvae is a little more involved. Some past practices include pulling up emergent plants and shaking the roots into a bucket or separatory cylinder. This is not generally satisfactory for surveillance because it requires pulling up plant species which are not always easy to dislodge from the substrate or taking large volumes of material back to a lab for sorting. Crans and Walker (1986) recommend use of the modified bilge pump, in conjunction with a soil sampling sieve. To sample for larvae, place the end of the bilge pump into the water at the base of a cattail or other host plant. Lower the pump into the root mat of the plant, but not directly into the mud layer. Draw water up the pump shaft, lift the pump out of the water and let it drain into the sieve. One can then count or collect the larvae in the sieve.

The hospital syringe is another useful tool in situations where the dipper is not feasible. Dredge spoil deposition sites can serve as sources of considerable mosquito production. When the soil within these sites becomes cracked and holds water, larval surveillance employing the standard dipper is difficult to carry out. Personnel of the Salem County Mosquito Extermination Commission have developed the technique of using a hospital syringe fitted with surgical tubing, which may be inserted between cracks (Kent *et al.*, 1987). Water and larvae are drawn up into the graduated syringe; thus, the presence or absence of mosquito larvae can be ascertained even if standard dipping procedures cannot be carried out.

Another device for collecting mosquito larvae from dipper-inaccessible habitats is a suction sampler, developed by Waters and Slaff (1987) for use in sites with small openings or shallow water. It consists of flexible plastic tubing feeding into a plastic, screw top drink bottle. Mouth suction applied to the upper tube draws water and larvae into the bottle. The larvae are then transferred from the bottle to a collecting container. This device can be easily constructed using inexpensive materials and also serves to demonstrate how valuable a little interest and ingenuity can be.

Identification of Samples

Much earlier, a definition of the term "larval surveillance" was given, which bears repetition. Larval surveillance involves the determination of the presence or absence of immature mosquitoes within a given site, and their density, but it does not stop there. It also refers to the collection and identification of these larvae, and some form of recording these data. The author does not wish to imply that one must collect and identify a sample from every site inspected and found to contain immature mosquitoes. During the course of preparing this paper, the 20 mosquito control agencies in New Jersey were surveyed. Four of the agencies collect a sample and identify the mosquitoes obtained from every area yielding a positive inspection. The information is used to document mosquito production as a justification for treatment with pesticides. It is also a good way of determining species distribution, density and seasonal occurrence within one's county, and is a means of evaluating control results. Not everyone, however, has the manpower available to do this on such an extensive basis. As a result, we propose that samples be collected and identified, not from every area inspected, but from a representative sample of areas. Collecting samples during the early season only, and assuming that the species composition will remain the same throughout the rest of the season, will not provide an accurate picture of the species and their seasonal occurrence within a county. If identification cannot be done immediately, the larvae can always be preserved and the ID work done during the winter months.

One of the advantages of routinely identifying collections from a variety of sites is the potential for decreasing the amount of pesticides applied, and thus saving money. If an area is found to only produce non-pest mosquitoes, treatment with pesticides is not required. One might think that the resultant savings are not significant enough to warrant the effort, but a study performed in Burlington County in 1980 revealed that 20% of airspray larval samples collected in this county did not require treatment (Smith and Gooley, 1980). This represents a significant savings of both time and resources.

Table 2 contains a list of mosquitoes found in New Jersey which are not known to be nuisance or vector species, and thus do not require treatment with pesticides. This list could possibly be expanded to include *Ae. abserratus* and *Cs. morsitans*, which are said to rarely feed on man and are not known to be involved in disease transmission (Carpenter and LaCasse, 1955).

The identification of larval samples is another reason why it is important for each county to have a surveillance specialist on staff. It is also very important that there be some form of training available to ensure a high level of competence in mosquito identification specialists. In addition, it is a good idea to keep a larval reference collection of species found within one's county, and to make this available to field personnel.

TABLE 2. New Jersey mosquito larvae which do not require treatment with pesticides

Species	Habitat
Cx. territans	Any permanent clean water, usually associated with vegetation
Or. alba	Treeholes, tires, containers
Or. signifera	Treeholes, tires, containers
Tx. r. septentrionalis	Treeholes, tires, containers
Ur. sapphirina	Permanent water with emergent or floating vegetation exposed to sunlight, especially duck weed
Wy. smithii	Pitcher plants

Management of Surveillance Data

Data management is an important part of any surveillance program. Why go to the trouble of inspecting, collecting samples and identifying them if no record of the results is made? The basic information collected with each sample should be: the date, location or site, type of habitat, climatic conditions, degree of cloud cover present, the larval or pupal density, stages present and the species (determined in the lab through identification). This represents the basic information to be collected; if a county wishes to include more than that, of course, it is their prerogative.

Previously, microhabitats within breeding sites were discussed. When dipping for larvae, each separate microhabitat within the site should be sampled. If a breeding site contains only one particular habitat type, then dipping should be carried out at several stations within the site. Three dips should be taken at each station. The importance of sampling from a number of stations within a breeding site cannot be overstressed. Larval inspections should never be confined to dipping from just one station, or microhabitat, within an area. Of course, it involves a little more work to learn to recognize the existence of microhabitats within a single site, and to dip from each of these, but it gives a much more accurate picture of the mosquito breeding activity within the site.

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.

Protecting Samples from Contamination

Contamination is a problem in agencies where collecting equipment has been contaminated with pesticides causing larvae to die before they can be properly processed. Common sense is the key to eliminating contamination from the laboratory. Do not carry dippers, or any other collection materials such as vials, bags, pipettes etc. in the vehicle compartment that is used to store and transport pesticides. Do not use a dipper to measure pesticides; always keep pesticides out of the lab area.

Basic Guidelines or Larval Surveillance

In summary, some very basic guidelines for larval surveillance include:

Organize breeding sites into some kind of system-route book, map system or index cards.

Train field personnel-they must be able to differentiate between mosquito larvae and mosquito-like larvae, and they should be able to associate mosquito species with their seasonal occurrence and preferred breeding habitats.

Use the correct technique when approaching the area to be sampled-avoid disturbance of the water, avoid casting a shadow onto the water.

Use the appropriate dipping technique for the habitat to be sampled and the species to be collected.

Identify all samples to species and instruct field personnel to assure that the samples are marked with date, location and habitat data.

Every county mosquito control agency should have a trained surveillance specialist on staff.

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The information on disease surveillance, vector surveillance and laboratory surveillance should be appropriately co-ordinated at district, provincial and central level into one data system. There needs to be an identified mechanism for flow of information and action that needs to follow DF/DHF information system should be an integral part of the overall communicable disease surveillance system. 3.3 Integrated vector control activities.Â 1. Development of clear guidelines for field level staff on vector control (adult and larval) annexure iii. 2. Larviciding in selected containers with appropriate larvicides. 3. Biological control using fish e.g. Poecelia reticulata (guppy) for water storage tanks/ ponds. surveillance includes dipping, breeding site identification and larval identification. Dipping is performed by taking a small amount of water using a sampling device called â€œdipperâ€ and examining the sample for the presence or absence of mosquito larvae. Adult mosquito surveillance is key to determining the type and density of mosquitoes present throughout the City and through their testing to determine the presence, location and intensity of WN virus. This report provides updated guidelines for evaluating surveillance systems based on CDC's Framework for Program Evaluation in Public Health, research and discussion of concerns related to public health surveillance systems, and comments received from the public health community. The guidelines in this report describe many tasks and related activities that can be applied to public health surveillance systems. INTRODUCTION. In 1988, CDC published Guidelines for Evaluating Surveillance Systems (1) to promote the best use of public health resources through the development of efficient and ef Read the MHSAL Larval Sampling and Larviciding Guideline Section (page 8) and review the template for tracking these activities. If you have any sampling/ treatment questions please consult the Program Coordinator or the Field Surveillance Coordinator. 2020 MUNICIPAL PLANNING DOCUMENT â€“ Larviciding package. Page 7. D: larval sampling and larviciding guidelines. Culex tarsalis Larval Habitat. Culex tarsalis has a wide range of habitats that includes grassland and open woodland areas, usually with warmer water areas exposed to the sun. In the spring, small numbers of Culex tarsalis larvae can us This report provides updated guidelines for evaluating surveillance systems based on CDC's Framework for Program Evaluation in Public Health, research and discussion of concerns related to public health surveillance systems, and comments received from the public health community. The guidelines in this report describe many tasks and related activities that can be applied to public health surveillance systems. No caption available. â€¦ No caption available.