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ENTOMOLOGICAL EVALUATION FOR PLANNING AND EVALUATING ANTI-LARVAL OPERATIONS

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Entomological evaluation constitutes an essential part of the information required for planning and executing the anti-larval operations. The development of an anti-larval programme needs the following entomological activities.

I Preliminary Investigations

II Collection of entomological baseline data

III Evaluation of the effect of the anti-larval measures on the vector population during the operations

I PRELIMINARY INVESTIGATIONS

These are carried out in two steps:

1. Assessment from existing information (publications, reports, unpublished documents) of the environmental conditions and meteorological data, the recognized and suspected vectors and their breeding potentialities and the dynamics of malaria transmission; response to anti-vector measures.

2. The collection of data for completing up-to-date information on anopheline vector distribution, preferential breeding places and resting places of vectors

and mapping of the area. Selection of the most productive breeding places and adult resting places as fixed capture stations for the collection of baseline data.

II COLLECTION OF THE BASELINE DATA

The objectives are the following:

1. To study the vector bionomics (if unknown) or to study only that part of the bionomics not well known and necessary for deciding on the anti-larval measures (breeding habits, speed of larvae development, dispersion and density of adult vectors during different seasons).

2. To establish the breeding potentialities and variations under the influence of the local environmental conditions existing in each geographically homogeneous area (if unknown).

3. To assess the susceptibility level of the local vector to the available adequate larvicide.

4. Small scale trial to find the optimum formulation, dosage and timing of the application of the larvicide.

III ENTOMOLOGICAL EVALUATION OF ANTI-LARVAL MEASURES

The objectives are the following:

1. To evaluate the immediate impact of the larvicides on the larval population in order to detect the operational failure.

2. To evaluate the impact on the adult mosquitoes and contact with man.

3. To check the susceptibility of larvae when it is suspected that a change of susceptibility level has occurred.

The main methods used for collecting data for evaluation consist of:

(i) larval sampling and (ii) adult sampling; there are two types of sampling collection in fixed capture stations and collection at random. Fixed capture stations are places where initially a high larval or adult density is encountered and where regular observations are requested by employing the same methods and techniques.

Larval sampling

The method and procedures will depend on the objectives. While deciding on the area, type of breeding place to be sampled, and methods of sampling, the following aspects should be taken into account:

(i) the behaviour of different species as far as breeding behaviour is concerned;

(ii) dispersion and distance of flight;

(iii) places of concentration of larvae and adults;

(iv) use and limitation of sampling methods.

Before starting investigations on the breeding places the area to be investigated should be mapped and divided into more or less concentric areas. The first area should consist of the inhabited part and a peripheric zone of about 50 metres wide. The second one is the area surrounding the first and having a radius of about 500 metres from the first zone. The third area extends for about one kilometre from the border of the inhabited area. If necessary, fourth or even fifth areas can be established. The width of each area is not fixed and will vary according to the normal dispersion of the mosquito species and the availability of breeding places. There are situations when the only existing breeding places might be located at a distance of two or even three kilometres. For practical purposes the investigations of the larval breeding places should start systematically from the central area towards the periphery. If the types of breeding places and distribution are uniform in all the above-mentioned areas, it is sufficient for the preliminary survey to investigate only area 1. Mainly in the case of intra- and periodomestic species the larval density will be higher in the favourable breeding places closer to the inhabited area.

The sampling of the larval density in an anti-larval project has to take into account the variations of the relative density in space and time in each type of breeding place. While carrying out the study of the presence and/or relative density of mosquito larvae, the following aspects should be considered:

(a) some mosquito species are found in a large variety of breeding places whereas others are limited to a very restricted type of breeding habitat;

(b) the density of larvae in the breeding places is not uniformally distributed, the larvae being concentrated in the breeding places in some spots more than in others. It is well known that the free surface of large breeding places does not have larvae, these being concentrated on the border or around floating vegetation if this is not in movement, and in shaded areas, and in spots where gravid females are attracted to lay eggs. Slow movement of the water surface produced by the wind may concentrate the larvae in a corner of the breeding place. Also the larvae will concentrate in shaded areas when they show a realtive negative phototropism, whereas larvae with a positive phototropism will concentrate in the sunny areas. (A few examples from the field are represented in Plate 1, Fig.1.2).

(c) the concentration of larvae in the breeding place might be produced by the reduction of the breeding place surface or a dilution of the larval density might be produced by torrential rains by washing the breeding places or by increasing the surface;

(d) the particular behaviour of larvae should be known otherwise these could escape collection. The example of A.funestus is classical - the larvae are sunk under the vegetation and remain attached there and very easily escape collection. The larvae and pupae of A.taeniorhynchus (Man.) live attached to the roots of floating aquatic plants (the pupae come to the surface a short time before hatching);

(e) it should be taken into account that the larval densities are relative and show only trends. The trends of the mosquito densities in an area are established by:

1. collection in fixed capture stations, and
2. collection at random.

The selection of fixed spots for regular larval sampling will be made after detailed preliminary investigation of existing breeding places and detection of the sites with higher relative larval density. Once the spot to be selected is located each breeding place will be investigated each time by the same methods and tool and preferably the same number or a multiple of the standard number of dips (it is understood that the number of dips will vary with the size of the breeding place). The selection of the tool for collecting larvae will depend on the type and surface of the breeding place as well as on the type of investigation. Hoof prints or very small breeding places will need a smaller collecting device than a breeding place in a pool, lake or river. Whereas in a hoof print we can practically collect an absolute number of larvae by three to four dips, such an achievement in a large breeding place is not possible. Therefore, for large breeding places a standard number of dips is used - a minimum of ten dips and if the density is low a multiple of ten dips for each capture station. It should be mentioned that large breeding places might have several capture stations as judged necessary by the investigator.

Collection of larvae for the study of the effect of larvicide and evaluation of operations

In practice the larval density after each round of application should be evaluated at 24-48 hours and/or the last day before the application of the larvicide. In special studies the larval density can be established during the same day before the application of the insecticide.

The significance of the larval stages will depend on the interval in days between the date of application of larvicide and the date of collection.

The presence of third/fourth stage larvae at 24-48 hours after larviciding will indicate that the larviciding has not been entirely effective. The importance of the eventual failure will depend on the proportion of breeding places found positive and larval density in the positive breeding places. The presence of larvae of an age indicating operational failure might be due to insufficient coverage, insufficient dosage, or insufficient penetration of the insecticide through the vegetation. The constant presence of larvae of stage I and II only, during the day preceding the application of the larvicide will indicate that the interval between each round of application could be increased by at least two or three days and that the insecticide has a residual effect. The presence of I/II and III stage larvae one day before larviciding will not indicate any failure of the insecticide. Even early IV stage larvae during the last day before larviciding does not indicate any failure providing that the density is low and that the classification of larval stages and density is accurately established (see: How to recognize the larval stages, Plate 2 Fig.1.4) and the efficacy of larviciding conferred by the study of the adult densities.

IDENTIFICATION OF THE LARVAL STAGES

It is well known that the mosquito larvae pass through four larval stages which can be easily recognized by taking into account dimensions and some elementary external morphological charactersitics. All stages can be easily recognized under field conditions with the naked eye or by using a hand lens of low power (X5).

The identification of the larval stages is necessary for the study of the duration of each larval stage and the evaluation of the efficacy of the larvicides.

In the following Table and Plate, the morphological characteristics are given which can be used in practice by every field worker.

Table No. 1

Macroscopic characteristics to be used for the identification of various larval stages of anopheline mosquitoes

Larval stage	Morphological characteristics	
	Length in mm	Other characteristics
I	1 1/2 - 2	Presence of a small sharp tooth projecting from the fronto-clypeus, midway between the eyes (egg bracker); collar large. Plate 2 Fig. 1
II	2 - 3	Collar narrow. Plate 2 Fig. 2
III	4 - 5	Collar large. Plate 2 Fig. 3
IV	5 - 7	Collar narrow. Plate 2 Fig. 4

All stage IV larvae are recognized due to their very developed thorax rectangular in shape (in anophelines). Pupae near to hatching in a few hours have the cepholotonax which is a darker brown than the rest (Plate 3 Fig. 1), whereas young pupae have a lighter colour (Plate 3 Fig. 2).

INVESTIGATION OF THE DENSITY OF ADULT VECTORS

Collection of adult mosquitoes in a larviciding programme is essential since the main objective of the larviciding is to reduce the adult mosquito density to an insignificant level.

The study of the adult mosquito density has two objectives:

1. To give information, directly or indirectly, on the influence of larviciding on man-mosquito contact.

2. To indicate, in the absence of larvae in sufficiently large areas or in naturally isolated areas, where infiltration of adults from untreated areas is excluded, if breeding has ceased or if there are still some spots which produce adult mosquitoes due to operational defects or other causes.

Selection and number of capture stations

While selecting the capture stations the following aspect should be considered:

1. Siting

Proximity to the productive breeding places. It is well known that the area surrounding the productive breeding places has the highest mosquito density.

2. Degree of Attractiveness

This degree is determined by investigating a number of mosquito resting places before the start of the larviciding operations during the main period of the mosquito development. Only those capture stations showing repeatedly the highest density will be selected (investigated two or three times at intervals of not more than five days). After Anti-larval measures have been implemented, it is possible that the small number of adult mosquitoes which might still persist in the area enter these attractive resting places due to their particular microclimate and attractiveness of hosts.

3. Number of capture stations

This depends on the extent of the area to be investigated, location and concentration of breeding places, possibility of carrying out extensive larval

ENTOMOLOGICAL INVESTIGATION IN A LARVICIDING PROGRAMME

Type of Activity	Objectives	Methods to achieve the objectives	Duration
Preliminary survey	To collect the necessary data for the planning of the collection of the entomological base-line data	1. Assessment of the existing information on vector distribution, breeding behaviour and prevalence, response to insecticides, etc. 2. Distribution and type of breeding places. 3. Larval survey in characteristic breeding places for different species completed by adult mosquito survey in the area of the most productive breeding places.	One or two months during the main season of vector development
Base-line data	1. To establish the breeding potentiality under local environmental conditions	1. Distribution surface dynamics of different types of breeding places (Seasonal variations of the: surface, maximum and minimum variations, the average of the surface (and eventually the volume for small breeding places) and larval density in representative spots)	One year or less depending on the existing information
	2. To establish the basic susceptibility of vectors to the available larvicides	2. Susceptibility tests (only for larvae)	
	3. To determine the formulation, dosage and interval of application of the available adequate larvicide.	3. Small scale trial including all representative breeding places (i) one week investigation of the larval densities of the breeding places to be treated and of those to be used as control, (ii) larval density started 24 hours after the application of each round of larvicide and carried out every second day in well selected capture stations and at random until the reappearance of stage IV in the most favourable breeding places. After the appearance of stage III larvae daily larval densities should be carried out	During the main transmission season. Minimum two months or during the whole season when the larvicideing is to applied on a large scale

Type of activity	Objectives	Methods to achieve the objectives	Duration
Evaluation of the operations of the larviciding programme	1. To evaluate the impact of anti-larval measures on the larvae population	(a) Larvae collection in fixed spot and at random - not earlier than 24 hours after the application of the larvicide and one day before the next round of larviciding; in practice larvae collection one day before larviciding round is satisfactory if the interval between each round has been correctly applied.	As long as the larviciding programme
		(b) Larvae susceptibility tests	Only when indication on the change to the susceptibility level to the larvicide used
	2. To evaluate the impact of the operations on the adult population	Adult collection in the representative capture station and/or at random using a fixed or irregular schedule. (Collection at 10-15 days' interval for several consecutive days when using traps)	As long as the larviciding programme but the extent of such investigations will depend on the real needs for this method

collection, etc. It might be limited to up to ten fixed capture stations depending on the size of the area.

Random collections are essential to complete the observations in fixed capture stations.

4. Timing of collection

This depends on the method of collection and the objective.

Methods of adult collection

1. Collection of indoor resting mosquitoes - pyrethrum spray collection, or hand collection; the application of one or another method depends on which of these methods is more efficient and easy to apply under local conditions (see use and limitations of this method).

Timing. Two weeks interval or at any other interval judge necessary for random collections and collection in the fixed capture stations.

2. Trap collection (exit trap in hut with modified eaves)

Applied to huts close to the breeding places with high density and attractivity before the start of operations. The window traps should remain in operation all the time and mosquitoes can be collected at one week intervals or during the day of application of the larvicide; no 24 hours survival rate is necessary.

3. Baited traps

A man or animal (depending on the attractiveness of such a host for a given species of mosquito) could be permanently used. If a man is sleeping inside, he could be protected against mosquito bites.

The trap should be located in the area neighbouring the breeding places. Observation could be made daily or at two weekly intervals for a minimum of three consecutive days. Both exit traps and baited traps are useful for collecting house frequenting mosquitoes (endophilic and exophilic mosquitoes coming to bite at night inside).

4. Light traps

CDC miniature light traps using block light or any other type of available trap could be used.

Siting of light trap. Inside houses (huts) which attract mosquitoes, neighbouring breeding places. Outside houses which are near breeding places, but very close to the entrances that the mosquitoes use to enter the houses. In an area where animals are present, very close to the place where the animals rest.

The light trap should be posted for several consecutive days at a minimum of two weeks interval. The attractiveness of the light trap for the local vector should be known since some species are less attracted than others.

Man biting collection off human baits in order to evaluate the man/vector contact

This method is applied (i) when other methods do not offer sufficient inforamtion, (ii) as a complementary way in which to check the results obtained by other methods, or (iii) when this is the only efficient method of collection, as in the case of some exophilic mosquitoes. Indoor as well as outdoor collections should be carried out.

Collection off animal baits (outdoor collection)

Remarks

1. The collection of adult mosquitoes in a larviciding programme is particularly important when the breeding places are very extensive and the larvae density cannot be investigated accurately. During the period when the vector density decreases normally to considerably low figures, the adult collections could not be of too much operationsl significance.

2. When the anti-larval operations are started, during the period when already the adult density is high, the regular investigations of the adult density in the area should start not earlier than two weeks after the start of operations, when the normal density will naturally decrease to low levels. If the study of parity rates after the start of larval operations shows the absence of nulliparous, will indicate that the breeding has been completely arrested, the presence of nulliparous females will indicate that the breeding is continuing.

3. It should be kept in mind that the longevity of the adult mosquitoes is not modified by anti-larval operations.

ANNEX

CLASSIFICATION OF THE LARVAL HABITAT
(Modified after Bates)

I LARGE OR MEDIUM SIZE HABITATS

A. Permanent or semi-permanent standing water (fresh or brackish water)

1. Marshes or marshy shores of lakes
2. Swamps
3. Ponds
4. Pools
5. Puddles
6. Seepages
7. Burrow pits
8. Wells

B. Running water

1. Springs
2. Streams
3. Rivers
4. Channels

C. Temporary breeding places

(a) Natural

1. Rain pools
2. Pools on river banks created by inundation
3. Puddles

(b) Artificial

1. Pools
2. Small irrigation water channels
3. Irrigated surfaces, rice fields
4. Surface ditches for the evacuation of waste water or for drainage of temporary waters

Form No. .....

LARVAL COLLECTIONS

Country .....
Region .....
Area .....
Zone .....
Section .....
Locality .....
Altitude* .....
Location on the map (No. ...

Unsprayed/sprayed area .....
Date of last residual spraying .....
Insecticide .....
Date of last larviciding .....
Larvicide ..... Quantity acre (ha)

Collection record :

Name of collector .....
Date of collection .....
Time of collection .....

Date (period)	Type of breeding place & approx. surface[1]	Distance from nearest house*	Water Characteristics: Quiet running	Water Characteristics: Clear turbid	Water Characteristics: Depth[2] approx.	Light[3]	Vegetation[4]	Method of collection[5]	Breeding places investig.: No.	Breeding places investig.: Pos.	No. of dips	No. of larvae[6]	Larvae per dip[6]	Species identified and No of larvae[6]: Tot.	per dip	Tot.	per dip	Tot.	per dip	Tot.	per dip

1. Spring, stream, river bed, irrigation canal, swamp-like seepage, hoofprint, rain pool, spring pool, spring well, artificial containers (others to specify)
2. Shallow (less than 1 meter deep), deep (more than 1 meter deep)
3. Sunlight, semi-shade, shade, deep shade
4. Emergent, floating, prevalent species if identified
5. Dipping, larval net, (others to be specified) and approximate investigated surface
6. In brackets, larvae of III and IV stage when detailed investigations are made
6. Calculated only for positive breeding places

*If the case

This form could be used in preliminary survey, collection of baseline data and evaluation of anti-vector operations, for recording the results of investigations of each breeding place, or the results obtained from several breeding places belonging to the same type.

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