Entomological field techniques for malaria control

PART I

Learner's Guide

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Entomological field techniques for malaria control.
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Preface

This training module on entomological field techniques for malaria control is in two separately bound parts. The first part is the Learner's Guide, containing technical information; Part II is the Tutor's Guide, which provides advice for those responsible for conducting training programmes.

The module is one of several[1] being produced by the World Health Organization, each concerned with a different aspect of the control of malaria. It can stand alone as a medium for the training of public health workers engaged in entomological field work for malaria control, or it can be used as part of a longer and more comprehensive training programme on malaria. The need for such a module was identified by the Member States of the World Health Organization, and it was developed by Mr J.L. Clarke in collaboration with the Programming and Training Unit of WHO's Malaria Action Programme. The original illustrations were meticulously prepared by Mr Yap Loy Fong of Kuala Lumpur, Malaysia. The text has been revised on the basis of observations and suggestions made by many people, in particular by Dr McWilson Warren, former Team Leader of WHO's Interregional Secretariat for the Coordination of Malaria Training in Kuala Lumpur, Malaysia, and by staff attached to that unit, notably Mr P. Blizard, Professor C.Y. Chow, Dr Han Il Ree, Professor B. Richter, Mr J. Storey and Dr Suwan Wongsarojana. The text was finally modified by Dr P.F. Beales, Dr C.W. Hays and Dr D. Muir.

WHO expresses appreciation to all who have assisted in the preparation of the module, and wishes to acknowledge the collaboration and financial support provided by the United States Agency for International Development in this and other activities of the Interregional Secretariat for the Coordination of Malaria Training.

[1] Already published: *Basic malaria microscopy* (1991).

Introduction

This Learner's Guide provides teaching material concerned with the entomological techniques involved in the control of malaria. Together with the related Tutor's Guide, it forms one component — or module — of a series of teaching materials on malaria. It is designed to be used throughout a formal period of training and provides information in a simple, easily understandable form.

The Guide is divided into chapters called Learning Units, which provide you with all of the essential information you will need in order to apply entomological techniques in the field. The tutor will discuss with you the tasks that you must be able to perform competently before your training is completed. Individual Learning Units deal with the theory underlying each task, and the necessary practical skills involved. Each Unit contains a set of learning objectives which identify what you should know and be able to do before you move on to the next Unit.

Your tutor will make use of the learning objectives to develop the content of the training programme and as a basis for assessing your achievements. You will find them valuable as a guide for your studies, both as a means of judging your own progress and as a summary of the key areas in which you can expect to be tested. For instance, if a learning objective states that you must be able to "collect indoor-resting mosquitos using a sucking tube" or "kill and preserve larval specimens", you may be certain that your skill in these tasks will be assessed.

Careful study of each Learning Unit in advance of related training sessions will help you to a better understanding of what the tutor is discussing or demonstrating. You may already have some knowledge or experience of some of the topics covered, in which case you will have more time to devote to studying material that is new to you. Many of the terms used in the text are explained in a section at the end of the Guide.

Because the information provided in the Learner's Guide is so comprehensive, you will not need to take many additional notes during your training. The Guide will also be a valuable source of reference after you have completed your training.

LEARNING UNIT 1

Basic tasks of collecting mosquitos in the field

Learning objective

By the end of this Unit you should be able to:

- define the basic tasks of mosquito collecting.

If you are to collect mosquitos in the field it is important that you know and understand what is expected of you. Your tutor will discuss with you the tasks involved in field collection, to make you aware of the responsibilities related to this work. You should feel free to ask questions about anything that is unclear to you.

Mosquito collection

Mosquitos are usually collected by a team of people working with communities, with other health workers, and with laboratory staff involved in the prevention and control of malaria. The team's activities consist of:

- collecting adult anopheline mosquitos and sending them to a laboratory for examination;
- collecting eggs, larvae and pupae of anopheline mosquitos and sending them to a laboratory for examination;
- keeping accurate, up-to-date records of collections in notebooks, and ensuring that the same information is also recorded on forms maintained by the team leader;
- constructing outdoor shelters and trap nets for the collection of mosquitos;
- maintaining collecting equipment in good order so that it is always ready for field work;
- ensuring that any damage to collecting equipment is promptly reported and quickly repaired;
- informing householders and village leaders of the reasons for collecting mosquitos and other specimens;
- answering questions from the community about malaria and referring those to which the answers are not known to the supervisor or team leader.

LEARNING UNIT 2

Malaria and its control

Learning objectives

By the end of this Unit you should be able to:

- define in simple terms what malaria is
- describe the life cycle of the malaria parasite and the mosquito, and relate this to the transmission of malaria
- describe what is meant by malaria control
- describe the kind of advice you will provide on vector control, to both individuals and communities.

The purpose of this Unit is to inform you about malaria and its control, so as to help you understand the reasons for the work you will be doing. Read it carefully and discuss it with the other learners. Your tutor will explain any points that are unclear to you. Reread the Unit on completion of your training so that you fully understand the necessity for malaria control and the purposes of your work.

The work you will be doing can be repetitive, hard and tiring, yet it is necessary if an acceptable level of malaria control is to be achieved. An insufficient understanding of the responsibilities of antimalaria work, especially the team work involved, can have adverse consequences for the health and welfare of people who live in areas where the disease occurs.

Malaria — the disease

Malaria is a serious disease and a significant health problem in most tropical countries. It is caused by a parasite which is found in the blood of people suffering from the disease. Symptoms of malaria include fever, chills, sweating and general body pains.

Malaria parasites are transmitted from one person to another by female mosquitos of the genus *Anopheles*, many kinds of which exist in different parts of the world. Only certain species of anopheline mosquito, known as *vectors* or carriers of malaria, can transmit the parasites.

When a mosquito bites, it sucks up blood. If malaria parasites are present in the blood, they are taken into the mosquito where they multiply. After several days, malaria parasites may be found (in the form of sporozoites) in the salivary glands of the mosquito. The mosquito also injects saliva when it bites, and can

thus transfer parasites to previously uninfected people. Moreover, the parasites multiply in the red blood cells and destroy them, causing anaemia. This is one of the reasons why people with malaria may develop a greyish-yellow skin colour.

A person who is sick with malaria can be bitten by many mosquitos every night, each of which can then carry the disease to several other people. Moreover, one mosquito can bite many times during its life, and a person who has been cured of the disease can be infected again if bitten by a mosquito carrying the parasites. This is why so many people can become sick with malaria.

A person who becomes infected with malaria can take medicine to kill the parasites. One of the medicines most commonly given is called chloroquine, but there are other antimalarial medicines that may be better in certain circumstances. It is important that people with malaria get the right kind of medicine from a community health worker or a doctor. If a patient does not take suitable medicine in the correct amount, at the proper time and for the specified number of days, the parasites in the blood will survive and multiply and he or she will become sick again.

People who have survived many attacks of malaria acquire some immunity to the disease, even when they take no medicine or an inadequate quantity of medicine. The number of parasites in the blood decreases and the infection may produce few, if any, clinical signs and symptoms. There may no longer be any risk of death from the disease. Consequently, in areas where malaria is highly prevalent, deaths from the disease occur mainly in children aged betwen 6 months and 5 years, who have had very few attacks. Nevertheless, a mosquito that bites a person who is resistant to the acute infection can still pass parasites on to other people.

Certain drugs can also be taken for prevention of malaria, and these are particularly important for workers responsible for collecting mosquitos, who may be bitten frequently. Such workers should take preventive medicine whenever necessary; trained health workers can prescribe the correct medicine and dosage.

The malaria parasite

The parasites that cause malaria belong to the genus *Plasmodium*. Four species of *Plasmodium* can live in the blood of humans and cause malaria: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. Usually only *Plasmodium falciparum* can cause death during an acute attack of malaria. Other kinds of malaria parasite infect animals but are not normally passed on to humans.

Malaria parasites are very small and can be seen only under a microscope. They can be observed by examining a drop of blood from an infected person. Sometimes there are numerous parasites, sometimes few. If there are only a few parasites, it may be necessary to spend several minutes examining a blood smear under the microscope before one can be found. Using the microscope, it is also possible to identify the species of parasite that is present: a trained worker can determine whether a patient has been infected by *Plasmodium falciparum*, or by the other, generally less harmful, species.

Life cycle of the mosquito

Malaria is transmitted to humans by female mosquitos of the genus *Anopheles*. Male mosquitos feed on plant juices and nectar, rather than blood, and therefore cannot transmit the disease. Moreover, only certain species of anopheline mosquito are successful vectors of the parasites. Some species prefer to take their blood meals from animals and thus transmit malaria to humans very rarely or not at all; some species do not live long enough to allow the parasites to multiply and develop inside them; and in some species the parasites seem to be incapable of development.

There are four different stages in the life cycle of the mosquito, of which the adult is the flying stage. The adult female anopheline takes a blood meal approximately every two or three days. This is necessary for the development of a batch of eggs, which are normally laid before a further blood meal is taken. The time required for digestion of the blood meal and development of the eggs varies with the temperature and humidity of the air.

Eggs are laid on water in batches of about 100–150. The sites for egg-laying vary from small amounts of residual water in such places as discarded food tins, coconut husks and hoof-prints, to larger areas of water in streams, canals, rivers, ponds and lakes. Each species of mosquito prefers a particular kind of water surface on which to lay its eggs.

Female mosquitos continue to lay eggs throughout their lives. Most lay between one and three batches, although in a few instances as many as 13 batches have been reported. Under the best conditions the average life of female anopheline mosquitos is about two to three weeks.

After two to three days, the eggs hatch and the mosquito larvae emerge. The larvae generally live just below the surface of the water (since they need to breathe air) and take food from the water. If disturbed, most larvae swim quickly towards the bottom of the water, returning to the surface soon afterwards in order to breathe; certain species, however, move rapidly sideways just below the surface.

There are four larval stages or instars. The time required for the different stages to develop depends on various factors, including water temperature: the development period is shorter in warm water than in cool water.

The small larva emerging from the egg is called the first instar. After one to two days it sheds its skin and becomes the second instar; at further intervals of about two days the third and fourth instars appear. At normal tropical water temperatures, the larval stage thus lasts about 8 to 10 days. Finally, the fourth instar develops into a pupa.

It is during the pupal stage, which lasts for two or three days at tropical temperatures, that the major transformation takes place, and the aquatic organism changes into an adult mosquito capable of flight. The pupa, shaped like a comma, stays on the surface of the water and swims when disturbed but does not feed. Its skin then splits and the adult insect emerges and eventually flies away. Mating soon follows; the female, which mates only once, normally takes its first blood meal after mating and then develops its first batch of eggs.

Most anopheline mosquitos bite during the night, some at sunset, others around midnight. Some enter houses to bite, while others usually bite outdoors. After biting, mosquitos usually rest for a short period. Indoor-biting mosquitos frequently rest on a wall, under furniture or on hanging clothes. Mosquitos that bite outdoors may rest on plants, in dark holes in the ground, or in other cool dark places.

Mosquitos may bite both people and animals. Host preferences differ between the species: some mosquitos prefer to take blood from humans, others take only animal blood. Clearly, those that prefer human blood are the more dangerous as they are more likely to transmit diseases, notably malaria. The incidence of malaria transmission may also vary with seasonal factors such as temperature, rainfall and humidity, all of which have a significant influence on the population of anopheline mosquitos.

Malaria surveys and surveillance

Information on the epidemiology of malaria is essential if the disease is to be controlled. Some may be acquired through malaria surveys conducted in the field with laboratory support, following a review of data from previous surveys and other relevant research. However, the information provided by a survey of this nature relates only to the situation at one particular time in a specific country or region. Continuing surveillance, on the other hand, provides a steady flow of information.

The information collected during surveys should provide answers to at least the following types of question:

- Is there malaria transmission in the country or region? If so, in which specific areas does it occur? What are the geographical limits (including limits of altitude) of the disease? Is malaria absent from large cities?
- What is the prevalence of malaria in the area and how severe is the disease? Is there falciparum malaria (the most dangerous form of the disease)?
- Is there evidence of *P. falciparum* being resistant to chloroquine and/or other antimalarial drugs?

For entomological purposes, a preliminary malaria survey should determine:

- which anopheline species are present;
- which of them are vectors of malaria;
- the biology and behaviour of adult vector mosquitos, including their resting habits indoors and outdoors, feeding habits, seasonal changes in the numbers biting humans, duration of adult life, and areas in which they are found;
- the breeding habits of the mosquitos;
- which vectors are susceptible or resistant to insecticides.

Vector control programmes for limiting the transmission of malaria in particular areas should be planned on the basis of this type of information.

A full malaria survey is needed only if data are unavailable, inadequate or unreliable. It may take several months or years to complete a full survey and this can be quite costly. Any such survey must be planned and directed by an

experienced malariologist, who can bring together information from parasitological and entomological services, together with data concerning the social habits, occupations and movements of the people, the types of houses they live in, availability of transport, climatic conditions, and the extent and distribution of health services. A partial or "updating" survey or a continuing (e.g. monthly) system of surveillance may be restricted to some of the items of a full survey, with a view to supplying missing data, assessing the results of any malaria control activities since a previous survey, or evaluating and/or replanning a programme.

Thus a limited survey, by means of malaria microscopy, may be needed to:

- determine changes in the number of malaria infections and in the proportions of the various kinds of causative parasite, with particular reference to *P. falciparum*;
- determine any variation in the effects of drugs used against *P. falciparum* infections.

For entomological purposes a limited survey may be necessary to:

- determine whether, following the use of vector control measures, there has been a reduction in the number of vectors biting people;
- determine whether there has been a reduction in the breeding density of vectors in the area;
- test the susceptibility of vectors to insecticides.

Once a control programme is in operation, regular observations are made to determine whether it is achieving its objectives. Mosquito collections are usually made at the same locations every two weeks, or at least monthly, so that any changes in vector numbers or behaviour can be observed and recorded. Random collections can also be made at other sites to confirm the results from regularly surveyed locations.

In some control programmes, spot checks or special parasitological and entomological studies are conducted. If there is a sudden outbreak of malaria in a village, such studies are often undertaken to determine why it occurred and what emergency measures against the vector and/or the parasite are needed to control the outbreak and prevent a recurrence.

Malaria control

In areas where many people have malaria it is important to lessen the burden on families and the community by treating the sick, preventing mosquitos from biting people, and possibly also killing mosquitos. In all areas, a combination of the first two approaches is needed to reduce the amount of suffering and disease. The third measure should be added whenever feasible in order to control the intensity and spread of malaria.

The treatment of malaria involves the use of specific drugs. Dosages for infants, children and adults vary according to body weight, but drugs are always given for a specified number of days. Giving too much of a drug can have unpleasant side-effects, but if too little is given there may be no effect on the malaria parasite. It is especially important that the proper treatment be given to people infected with *P. falciparum*: without it, they may die. Drugs may be given by

various people, such as village health workers, dispensary health workers, hospital doctors, trained members of health staffs, trained volunteers, or members of malaria teams.

Administration of drugs to prevent people from becoming infected with malaria is called malaria prophylaxis. Prophylaxis is normally restricted to specific groups of people and given for limited periods of time. If prophylaxis is continued for long periods in a population, *P. falciparum* in the area may become resistant to the drug or drugs being used, which could deprive the health services of their only means of curing severe infections and result in deaths. Furthermore, drug prophylaxis is expensive and full coverage is difficult to sustain for extended periods.

In addition to the treatment of malaria, much can be done to reduce the number of anopheline mosquitos by eliminating breeding sites and killing larvae, pupae and adult insects.

Breeding sites can be eliminated by:

- drainage or filling of areas where water collects;
- modifying the preferred habitats of particular vector species, for example by clearing streams so that the water flows faster and areas of vegetation are eliminated.

Larvae can be killed at breeding sites by spreading oil or other substances on the water or by introducing fish that eat the larvae.

Spraying houses internally with insecticide is the principal means of killing adult mosquitos that bite indoors. DDT is generally used for this purpose provided that the anopheline mosquitos that carry malaria in the local area show no significant resistance to the insecticide. If, having bitten someone who has malaria, a mosquito then rests on a wall sprayed with insecticide, it is killed and thus cannot transmit the disease to other people. This is why, in many countries, houses are sprayed every six months with DDT or every three months with a less persistent insecticide.

After several years of spraying with DDT or other insecticides, mosquitos and other insects may develop a capacity to survive contact with one or more of them. This insecticide resistance may develop slowly or quickly and range in degree from slight to total. It is important to know when resistance develops in one or more vector species because it may result in the loss of effectiveness of an insecticide in the control of malaria transmission. It is possible to measure both developing and established resistance, and this can help in deciding whether an interruption of spraying or a change of insecticide is required.

Various types of personal protection can be used to prevent biting by mosquitos that carry malaria. These include:

- the use of untreated or insecticide-impregnated bednets at night;
- the use of mosquito repellents or mosquito coils;
- the fixing of screens on windows to prevent mosquitos from entering houses;
- the use of insecticide-impregnated curtains on windows or doorways and/or in gaps between the roofs and walls.

LEARNING UNIT 3

Role of entomological work in malaria control

Learning objectives

By the end of this Unit you should:

- understand entomological surveys and monitoring and the purposes of these activities
- know how to prepare and maintain accurate records of all such activities.

Entomological services have several important roles to play in malaria control, including the following:

- identification of the vectors responsible for transmission of the disease;
- provision of basic information on vector species for planning purposes;
- monitoring the impact of control measures (for example, by determining changes in vector population density, rates of infection, susceptibility of vectors to insecticides, and residual effects of insecticides on treated surfaces);
- contributing to the investigation of problem areas where control measures prove unsuccessful;
- assisting in training.

When a control programme uses anti-vector measures, the first indicators of its effects can be monitored by carefully selected entomological observations. Reductions in vector density, parous rates and other parameters point to successful control. Further evidence of the success of malaria control may be obtained by malariometric spleen and parasite surveys and routine epidemiological observations.

It is important for all results to be accurately recorded and for at least two copies to be kept separately in case one is lost. Additional copies are required for transmitting data to other appropriate sectors of the health system.

Types of mosquito survey

Mosquitos brought to the laboratory are obtained in different kinds of field survey, each of which has an important role in the planning, operation or evaluation of anti-vector programmes.

Preliminary surveys

Preliminary surveys are the original, basic, short-term surveys used to gather baseline data for planning anti-vector measures. They provide information on

the following matters for each locality:

- the anopheline species present: specimens are obtained in the field and identified in the laboratory;
- determination and/or confirmation of vectors: specimens obtained in the field are examined in the laboratory for infection with parasites;
- the ecology of adult vectors, with reference to such factors as their resting habits, feeding habits (as determined by human-vector contact studies, and identification of blood meal sources), seasonal prevalence, and longevity;
- larval habitats, with reference to the types of water accumulation used as vector breeding sites, in relation to their size, duration, movement, chemical and physical nature, associated animal and plant life, exposure to sunlight, etc;
- susceptibility tests, intended to measure the sensitivity of vector species to available insecticides and to facilitate the selection of the most cost-effective insecticide.

Regular or trend observations

These are routine or long-term observations, and they are sometimes called operational surveys or monitoring. Observations are carried out regularly, e.g. weekly, fortnightly, monthly or half-yearly, for the purpose of evaluating the impact of control measures. They should provide information on the following matters:

- reductions or increases in vector density;
- changes in vector biology and behaviour, with reference to survival, feeding habits, resting places, degree of man–vector contact, etc;
- numbers and proportions of vector species with sporozoites in their salivary glands;
- proportions of vectors caught in sprayed houses which survive for 24 hours;
- susceptibility of vectors to insecticides.

Spot checks

Spot checks, which may include selected items from the regular observations listed above, are carried out in localities chosen at random. Since the fixed stations often used to monitor mosquito populations may not be representative of all areas, spot checks may be conducted randomly in selected areas to supplement routine observations or obtain a clearer indication of the effects of control measures.

Foci investigations

Foci investigations, which are similar to those outlined above, are undertaken in areas of new or persistent malaria transmission to determine why there is transmission or why the disease is not responding to the measures being applied, and to identify the best approaches to control.

LEARNING UNIT 4

Recognition of anopheline mosquitos

Learning objectives

By the end of this Unit you should be able to:

- distinguish mosquitos from other insects on the basis of their external characteristics
- distinguish between male and female mosquitos
- distinguish female anopheline mosquitos from female culicine mosquitos
- distinguish between the eggs of mosquitos and those of other insects
- distinguish between anopheline and culicine eggs
- distinguish mosquito larvae from the larvae of other insects
- distinguish between anopheline and culicine larvae
- distinguish mosquito pupae from the pupae of other insects
- distinguish between anopheline and culicine pupae.

The collection of adult mosquitos and of their eggs, larvae and pupae forms a major part of entomological field work. In the places where you will typically undertake collection, you are likely to find many other types of insect as well as mosquitos. You must therefore be able to distinguish between mosquitos and other insects. More importantly, since malaria is transmitted to humans only by mosquitos of the genus *Anopheles*, it is essential for you to be able to distinguish between anopheline and culicine mosquitos. You must also be able to identify anopheline mosquitos at all stages of their life cycle — eggs, larvae and pupae, as well as adults.

Recognition of adult mosquitos

The adult is the easiest stage to identify in the life cycle of the mosquito. Fig. 1 shows the main parts of the adult mosquito, and you should learn the names of these. The body, as in all insects, is divided into head, thorax and abdomen.

Three characteristics can be used to distinguish adult mosquitos from other insects:

- there is only one pair of wings
- there is a long proboscis (the tubular mouthparts)
- the body is covered with scales.

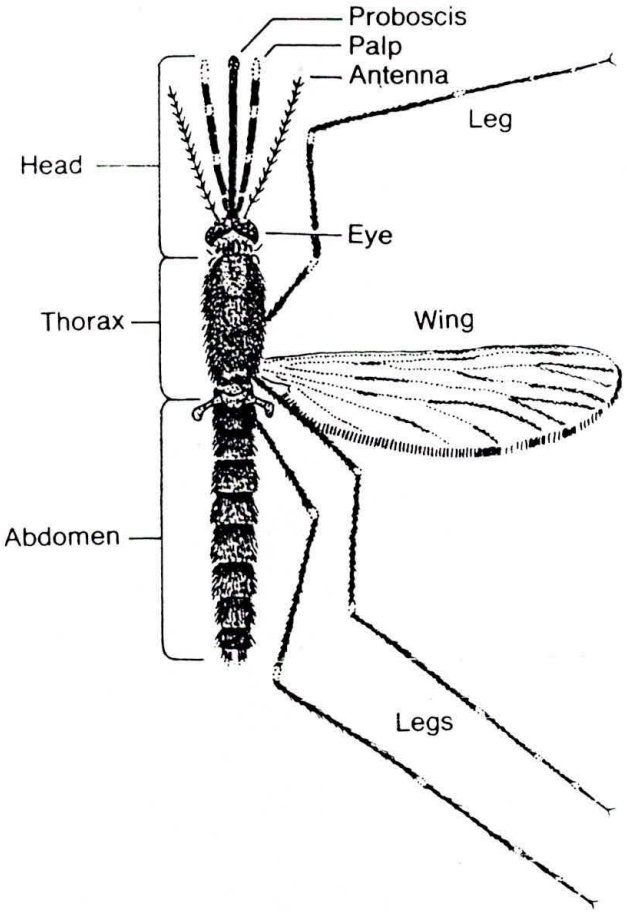


Fig. 1. **A mosquito**

Fig. 2 illustrates a number of common insects. On the drawing of the adult mosquito, note particularly the long mouthparts (proboscis), the single pair of wings, and the three pairs of comparatively long legs.

Distinguishing female mosquitos from males

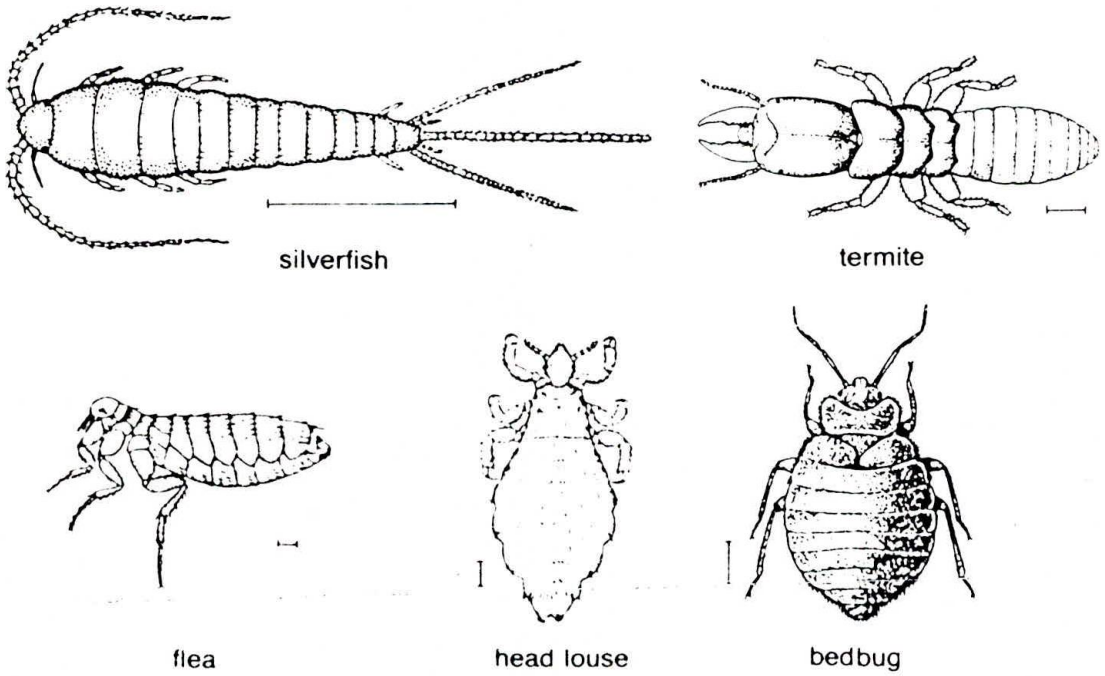
Because only the female mosquitos take blood meals and transmit malaria, it is females that you will be required to collect in the field. It is therefore important to be able to distinguish female mosquitos from males.

The male has very long hairs on the antennae, which consequently have a bushy appearance, rather like a moustache. On the antennae of the female the hairs are few in number and short. You should compare the male and female mosquitos illustrated in Fig. 3.

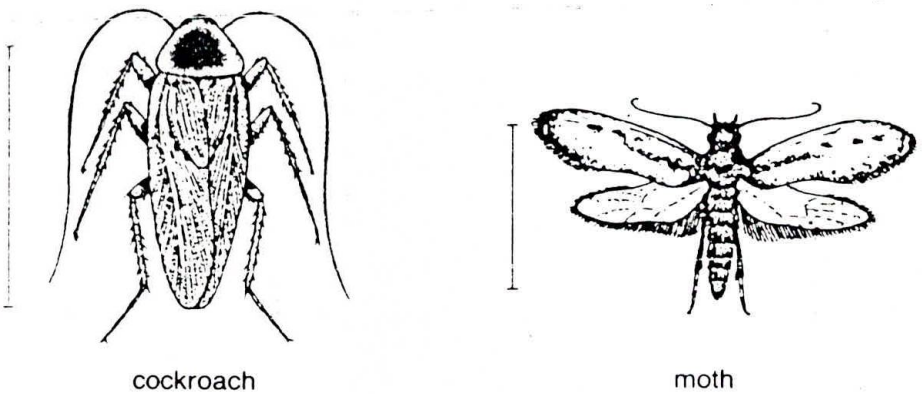
Distinguishing female anopheline mosquitos from female culicines

With live mosquitos, you can distinguish between anopheline and culicine mosquitos by observing their resting postures. Anophelines rest at an angle between 50° and 90° to the surface whereas culicines rest more or less parallel to the surface. These resting postures are shown in Fig. 4.

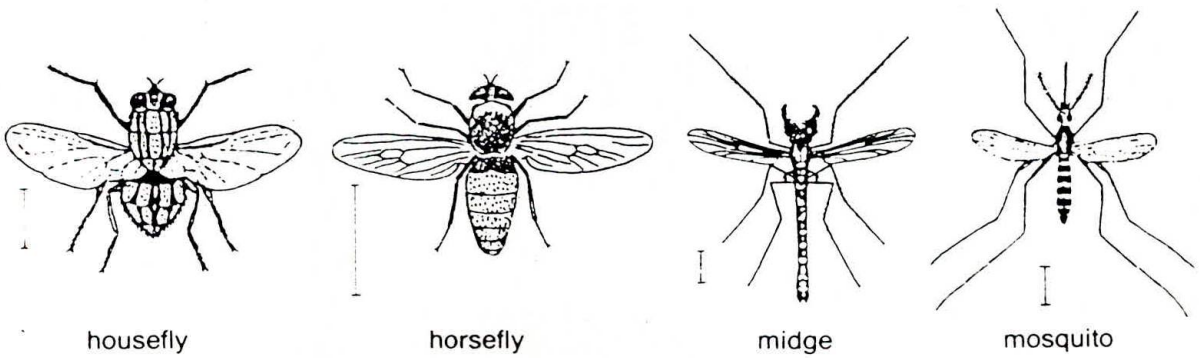
A. No wings



B. Two pairs of wings



C. One pair of wings



Note: Line scales indicate actual lengths of insects.

Fig. 2. **Common insects**

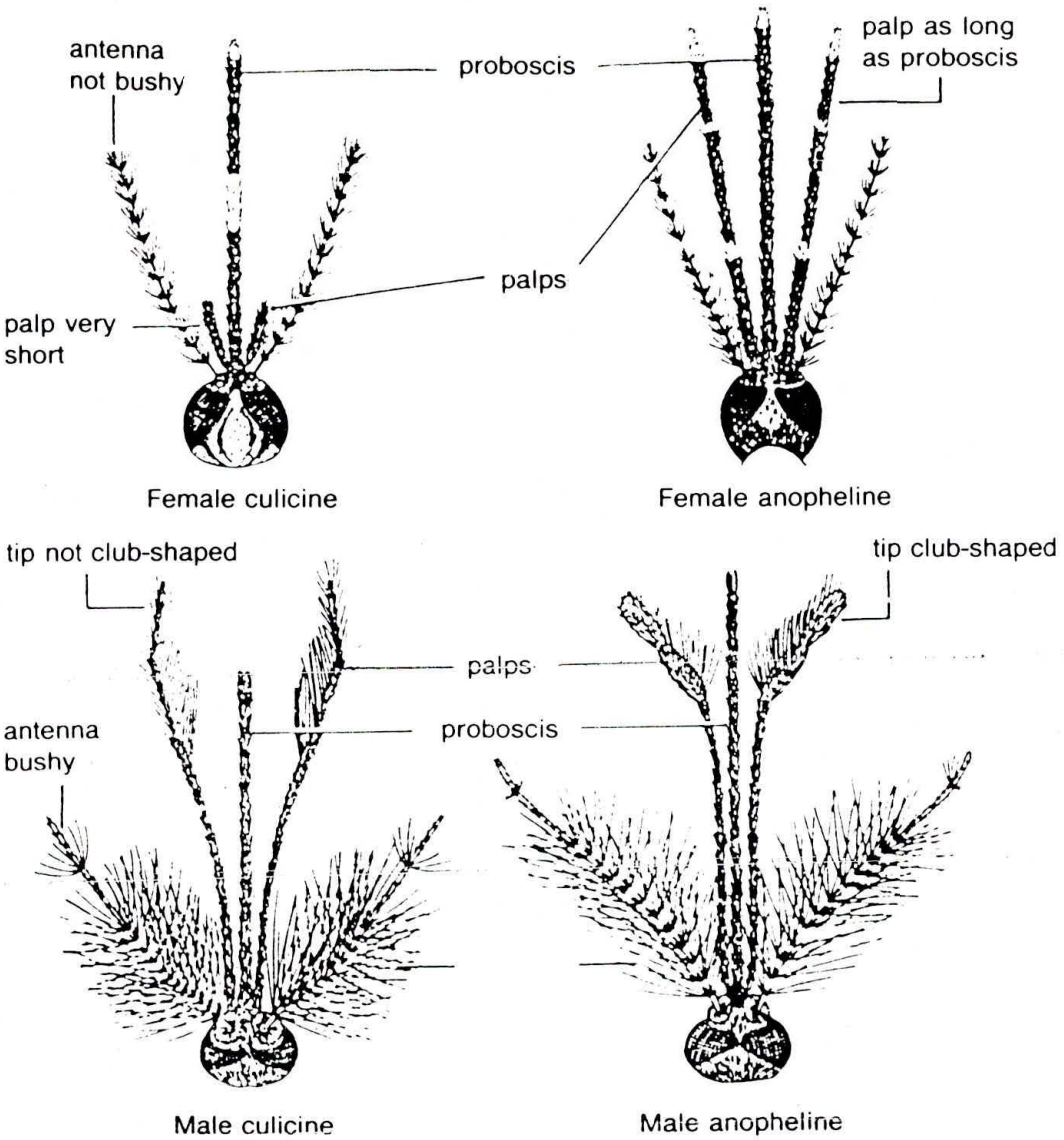


Fig. 3. **Heads of male and female anopheline and culicine mosquitos**

Anopheline mosquitos can also be distinguished from culicines by the length and shape of the palps. The differences, which can be seen in Fig. 3, are as follows:

Female: anopheline — palps as long as proboscis
culicine — palps very much shorter than proboscis.

Male: anopheline — palps as long as proboscis, club-shaped at tip
culicine — palps longer than proboscis, with tapered tips.

Distinguishing mosquito eggs from those of other insects

The eggs of anopheline mosquitos and of some culicines float on the surface of water; those of other insects do not float but are found attached to plants or under stones or other objects. The eggs of some *Aedes* mosquitos may be found in places that are liable to dry up; they can survive those conditions for some time.

Distinguishing between anopheline and culicine eggs

To distinguish between anopheline and culicine eggs you should observe whether they clump together in a "raft" (*Culex*) or float separately (*Aedes*), and whether they have "floats" (*Anopheles*). It is important that you learn these differences, which are illustrated in Fig. 4.

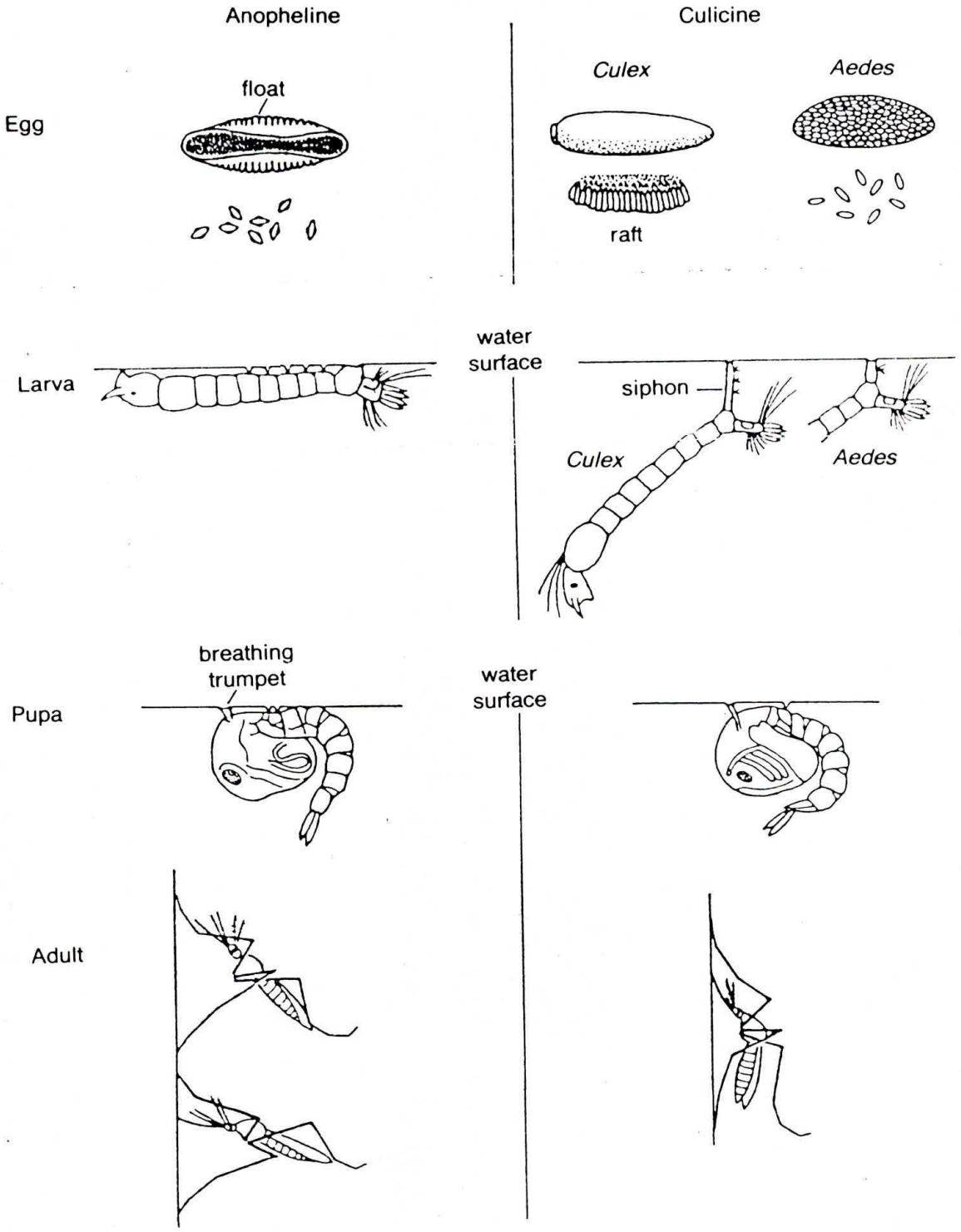


Fig. 4. **Life cycles of mosquitos**

Distinguishing mosquito larvae from the larvae of other insects

Mosquito larvae differ from the larvae of other insects in the following respects:

- they remain just beneath the surface of the water (because they need to breathe air through a special breathing tube);
- the head is narrower than the thorax.

Distinguishing between anopheline and culicine larvae

In the culicine larva the breathing tube is expanded and extended to form a "siphon". In the anopheline larva there is no siphon.

In Fig. 4 you will see that the siphon allows the culicine larva to hang down some distance from the water surface, whereas the anopheline larva rests parallel to and immediately below the surface.

You can therefore distinguish anopheline from culicine larvae because:

- the anopheline larva has no siphon and rests parallel to the water surface;
- the culicine larva has a long siphon and hangs down from the water surface.

Look again at Fig. 4 and study these differences carefully.

Distinguishing mosquito pupae from pupae of other insects

You must be able to distinguish mosquito pupae from those of other insects. Mosquito pupae, shown in Fig. 4, have the following characteristics:

- they are comma-shaped
- they hang just below the water surface
- they swim actively when disturbed.

Distinguishing between anopheline and culicine pupae

It is relatively difficult to distinguish anopheline from culicine pupae in the field. You should therefore collect all mosquito pupae and take them to the laboratory for identification. As can be seen in Fig. 4, the breathing trumpet of the anopheline pupa is short and has a wide opening, whereas that of the culicine pupa is long and slender with a narrow opening.

LEARNING UNIT 5

Hand collection methods and transport of adult mosquitos

Learning objectives

By the end of this Unit you should be able to:

- identify and assemble the parts of a sucking tube
- prepare paper cup containers for collections
- collect and transfer mosquitos, using a sucking tube
- label paper cups and make records of collections, accurately and completely
- collect mosquitos and transfer them to a paper cup, using a test-tube
- keep mosquitos alive in paper cups under field conditions
- transport live mosquitos from field to laboratory
- construct a killing tube
- demonstrate the proper care and cleaning of equipment.

One of the principal activities in entomological work is the field collection of mosquitos and their transport to the laboratory for identification and processing. As an introduction to field collecting techniques, this Unit will teach you how to collect and handle adult mosquitos without damaging them; how to keep mosquitos alive for a period of time in either the field or the laboratory; and how to transport mosquitos safely, without loss or damage, from the field to the laboratory.

At the end of this Unit you will make a killing tube in preparation for field practice in the collection of mosquitos.

The mosquitos you collect provide the main data for the entomological evaluation of malaria control programmes. Adults are collected in order to determine the species present in various locations and their relative density, to evaluate the impact of control measures, to study mosquito behaviour, and to assess seasonal changes. Adult mosquitos are also used to study malaria infection rates and mosquito age distribution, and to perform susceptibility tests. The objectives for each method of collecting adults are given in the relevant Learning Unit. It is most important for you to become proficient in all the collecting methods that you will use.

It is frequently necessary for mosquitos to be kept alive for delivery to the laboratory, so that they can be dissected: dead mosquitos become too brittle

for dissection. The laboratory will:

- dissect out the salivary glands and gut and examine them for evidence of infection with malaria parasites;
- dissect out the ovary and examine it in order to determine the parous rate;
- carry out susceptibility tests;
- determine the 24-hour mortality rate for mosquitos collected from sprayed houses.

In this first Unit of the practical part of your training, the main emphasis is therefore on collecting mosquitos by hand and keeping them alive until they reach the laboratory. The techniques that you will be taught involve the use of three pieces of equipment:

- sucking tube
- test-tube
- killing tube.

In the interest of brevity we shall refer, for the most part, simply to "mosquitos", it being understood that most of your collecting will in fact concern *Anopheles* and that the most important information required will relate to the species that carry malaria from person to person.

Essential equipment

The following equipment is needed for your activities: sucking tube; torch (with spare bulb and batteries); unwaxed paper cups; mosquito netting; cotton wool; mosquito cages; test-tubes; rubber stoppers for test-tubes; rubber bands; insulated picnic box; chloroform; trays; beakers; towels; pencil; notebook.

Test-tubes should normally be 150 mm long and 16 mm in diameter, but smaller ones (100 mm × 10 mm or 60 mm × 10 mm) may also be used. Small tubes are useful for collecting single specimens or when specimens are to be kept for some time before being examined and processed.

Identifying and assembling the parts of a sucking tube

The type of sucking tube illustrated in Fig. 5 consists of four parts:

- a length of glass or plastic tube
- a disc of wire mesh incorporated into the tube during manufacture
- rubber or plastic tubing, 80–100 cm long
- a plastic mouthpiece.

A sucking tube can be made locally using glass or transparent plastic tube. Wire mesh between the collecting tube and the rubber tubing is necessary to keep the mosquitos in the collecting tube and prevent their being sucked into the mouth and swallowed. (*Precaution*: plastic tubing should *not* be exposed to chloroform, which makes it lose its transparency.)

Sucking tubes and test-tubes should be kept clean. If they become dirty, clean them with wet cotton wool and ensure that they are perfectly dry before use. They should be carried in a way that will prevent breakage.

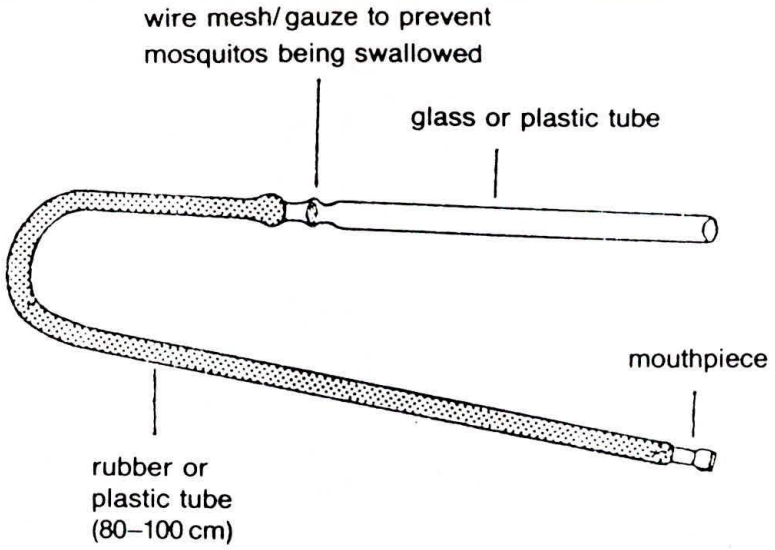


Fig. 5. **Sucking tube or aspirator**

Preparation of paper cups as containers

Cups made of unwaxed paper are very suitable containers for holding and transporting live mosquitos with minimum mortality. When these are bought in bulk they are very cheap and can be frequently replaced. Never reuse cups that have held mosquitos collected from sprayed houses, as residues of insecticide may kill mosquitos that you want to keep alive.

A paper cup container is made easily as shown in Fig. 6. It consists of:

- an unwaxed paper cup (capacity 250–300 ml)
- a square of mosquito netting with a hole for insertion of the sucking tube
- a rubber band
- a piece of cotton wool as a stopper.

If suitable paper cups are not available, small mosquito cages can be used or containers can be made from other locally available materials.

It is important to ensure that the hole in the netting over the cup is only large enough for the sucking tube to be inserted. If this hole is too large the mosquitos can easily escape or become damaged in the attempt.

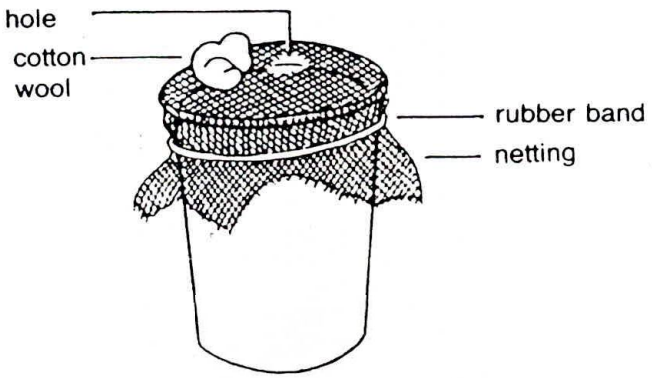


Fig. 6. **Paper cup prepared as a mosquito container**

Use of the sucking tube

When you wish to collect a mosquito and transfer it to a paper cup by means of a sucking tube, proceed as follows:

- With the mouthpiece in your mouth, hold the sucking tube with its opening 1–2 cm away from the mosquito.
- Move the end of the sucking tube closer to the mosquito and, at the same time, suck gently but quickly so as to draw the mosquito into the tube.
- Place your finger over the tube to prevent the mosquito from escaping.
- Place the end of the tube, with your finger still in position, near the hole in the mesh covering the paper cup. Remove your finger and quickly put the tube into the hole.
- Blow gently into the mouthpiece so as to transfer the mosquito to the paper cup; at the same time, tap the tube with your index finger to disturb resting mosquitos.
- Use the same technique to transfer mosquitos between different containers.

Be careful not to suck or blow too hard, as mosquitos are fragile and can easily lose legs or be otherwise damaged. Do not collect more than five mosquitos in the sucking tube before transferring them to the paper cup; if they collide with one another or with the tube they are likely to be damaged. Identification of species is important and may be hampered if specimens are damaged.

Labelling paper cups and recording collections

Accurate and complete labelling of paper cups is extremely important. During a field trip, mosquitos are collected at many sites. In the laboratory it is impossible to know the sources of collections if cups are unmarked or not labelled completely and correctly. Labelling should be done in soft pencil.

A record must also be kept in your notebook or on a field form of all collections made during a field trip. Particular attention should be given to keeping a record of all non-productive collecting efforts as well as of collecting that yields mosquitos.

The data you should record will vary with the purpose of collecting, but the minimum essential information is the following:

- location
- date and time of collection
- type of structure (house, stable, outdoor shelter, etc.)
- whether the structure has been sprayed and, if so, when this was last done
- your name.

Collecting mosquitos using a test-tube

A test-tube can be used to collect mosquitos by the following method, which is illustrated in Fig. 7.

- Hold the mouth of the tube directly over the mosquito.
- When the mosquito is disturbed it will fly into the tube.
- Close the mouth of the tube with your index finger or thumb.
- Remove your finger and push a plug of cotton wool into the tube.

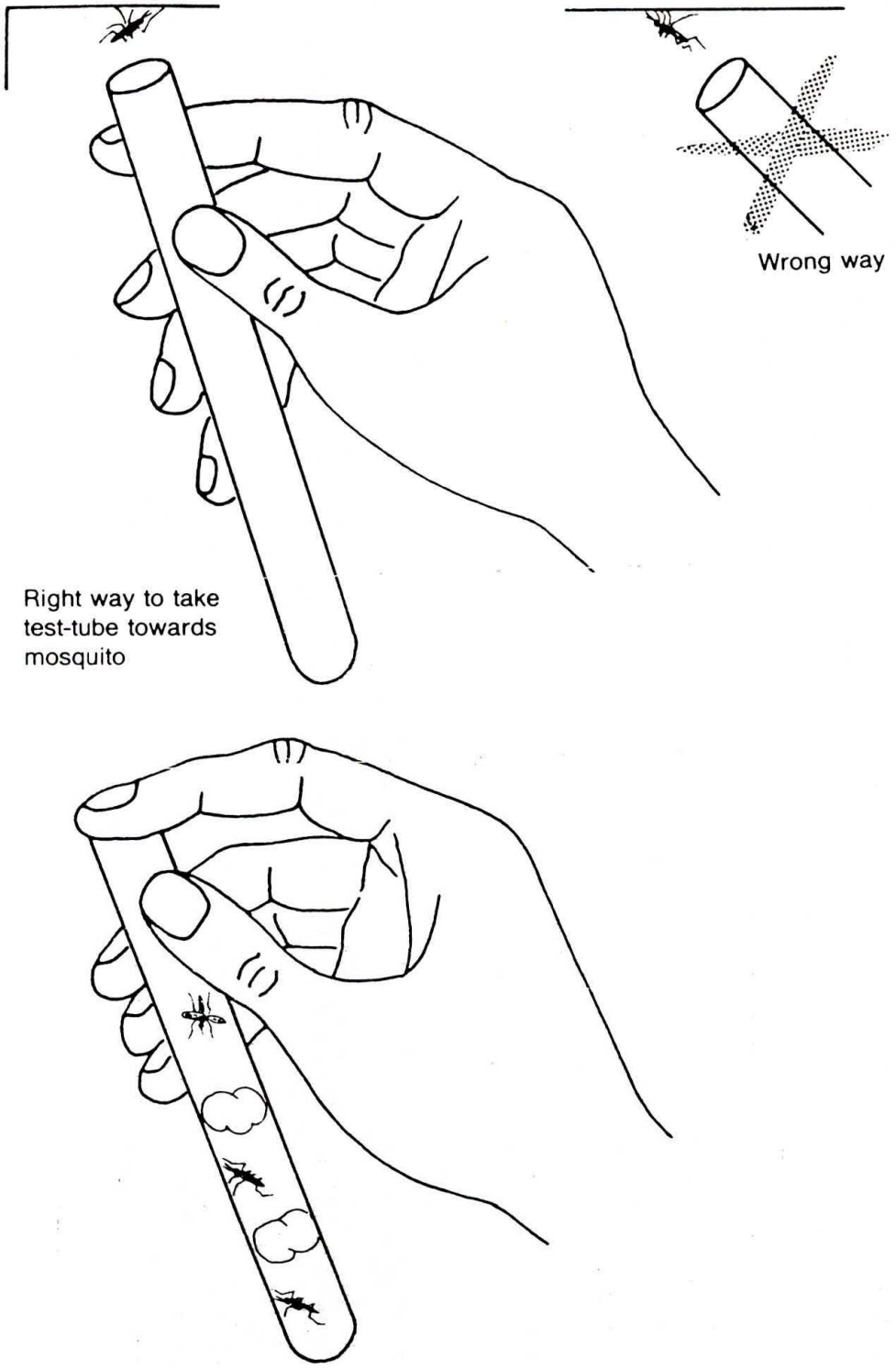


Fig. 7. **Using a test-tube for mosquito collecting**

- Push the plug down until the mosquito is trapped in the bottom 2 cm of the tube.
- Collect a second mosquito as described above and insert a second plug to trap it in the next 2 cm of the tube. In this way, several mosquitos may be collected in one test-tube.
- Record on a slip of paper all the data described above. Put the paper in the tube.

Keeping mosquitos alive in the field

Field collections must be sent to the laboratory as soon as possible. If mosquitos are to be kept for some time in the field, precautions must be taken to keep them in good condition. Remember that dead mosquitos dry out and become brittle, and cannot then be dissected.

In order to keep mosquitos alive in paper cups it is necessary to take the following steps:

- Soak pieces of cotton wool in 5–8% sugar solution.
- Squeeze out any excess sugar solution and place the cotton wool over the tops of the cups.
- Place cups holding mosquitos upright in a deep tray, a cardboard box or, preferably, an insulated picnic box.
- Cover the cups with a damp towel. Keep the towel damp until the mosquitos reach the laboratory.

If mosquitos have to be kept in the field for some time and an ant-proof container such as an insulated picnic box is not available, they can be maintained in the cups as shown in Fig. 8.

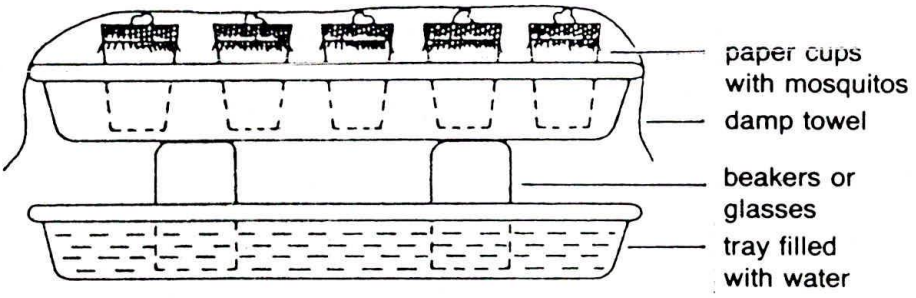


Fig. 8. **Keeping mosquitos alive in the field**

Make sure that you keep your mosquitos in places that are free from insecticide contamination.

Transport of live mosquitos

Adult mosquitos collected in the field have to be transported to the laboratory for examination. It is your responsibility to make sure that they arrive alive and undamaged.

Mosquitos can be transported safely over long distances in unwaxed paper cups provided you pack them as follows:

- Cover the cups with damp cotton wool.
- Place the cups upright in a box, preferably an insulated picnic box.
- Pack newspaper or other material between the cups to minimize movement.
- Place a damp towel over the cups.
- Close the box as tightly as possible to prevent loss of moisture.

During the period of transportation, it is essential to make sure that the mosquitos are not left in a vehicle with the doors and windows closed, or in direct sunlight.

Constructing a killing tube

A killing tube, as illustrated in Fig. 9, can be used to collect mosquitos for taxonomic studies or for other purposes not requiring live specimens. It is made from an ordinary test-tube in the following manner:

- Place pieces of cut rubber band at the bottom of a test-tube.
- Cover the rubber bands with a thin layer of loosely packed cotton wool and add a circle of filter paper.
- Pour chloroform into the tube to soak the rubber.
- Close the tube tightly with a rubber stopper to prevent evaporation of the chloroform; pour off excess chloroform the following morning.
- When the tube ceases to be effective, soak the rubber with chloroform again.

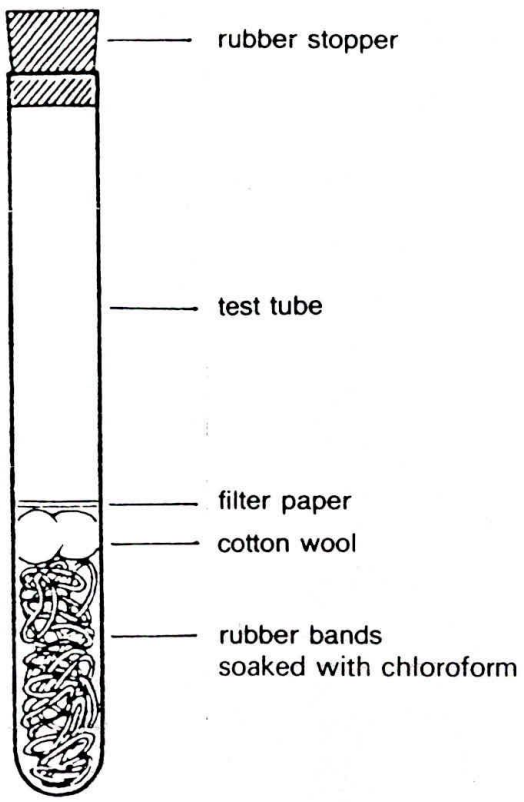


Fig. 9. **Killing tube**

Cleaning and care of equipment

It is essential to keep all equipment:

- free from dirt
- free from deposits made by mosquitos
- free from insecticide contamination.

Your sucking tube must be cleaned frequently and thoroughly. Its component parts should be separated and washed with water and detergent. The inside of the glass can be cleaned with damp cotton wool on the end of a thin rod. Each part must be completely dry inside before the sucking tube is reassembled.

Paper cups are inexpensive and should be regularly replaced. They must always be thrown away after use in insecticide-treated areas. The mosquito netting covers for the cups should be washed and dried.

The containers used for transporting the paper cups should be regularly washed with detergent. If cardboard containers are used they should be regularly replaced.

LEARNING UNIT 6

Hand collection of indoor-resting mosquitos

Learning objectives

By the end of this unit you should be able to:

- explain to householders your reasons for asking to enter their property
- use a sucking tube to collect indoor-resting mosquitos
- observe the normal resting places of mosquitos in houses and associated buildings
- accurately and completely label paper cups and prepare records of collections
- prepare collections and transport them to the laboratory.

During this Unit of your training programme, you will go out to the field and meet the local community for the first time. The methods of approaching people and explaining your intended work to them will be described to you in advance.

You will have the opportunity to practise the collecting methods you have learned in the laboratory and to observe the normal daytime resting places of mosquitos. You will also prepare mosquitos and transport them to the laboratory.

Many of the anopheline species that are malaria vectors rest indoors during the day and after biting. Hand collecting provides the following important information about these mosquitos:

- their usual resting places
- their distribution on different types of surface
- their indoor resting density
- seasonal changes in their density.

Hand collecting can also provide live specimens for:

- susceptibility and bioassay tests
- the production of eggs
- observations on mortality among insects from insecticide-treated houses
- taxonomic studies
- laboratory studies on rates of infection and survival.

Information on density obtained by hand collecting is less reliable than that derived from pyrethrin spray sheet collecting, which will be described in Learning Unit 7.

Essential equipment

In order to carry out hand collection in the field you will require the following equipment: a sucking tube; a torch (with spare bulb and batteries); paper cups with net covers; cotton wool; a pencil; and a notebook.

Your personal equipment should always be maintained in good order and kept in a bag ready for the field. You must pack glass items carefully so that they do not get broken while you are travelling.

You will also need the following additional equipment for the transport of specimens from the field: insulated picnic box (or other suitable container); sugar solution (5–8%); paper cups with net covers; cotton wool; towels; and newspapers to serve as packing material.

Communicating with village leaders and householders

Most of your mosquito collecting will be done in and around houses. It is therefore essential that you establish and maintain good relations with householders, village leaders and other members of the community. Preliminary discussions between village leaders and supervisors will be held before collecting starts.

You must ask permission to enter each property in which you desire to collect mosquitos. The tact needed and the method of approaching householders will be discussed with you by your course tutor. The following are some basic requirements.

Do:

- observe local customs (e.g. removal of shoes before entering houses)
- respect places of worship and shrine rooms
- respect the privacy of householders
- give any assistance you can to the villagers (e.g. provide advice on mosquito control and personal protection measures).

Do not:

- make excessive noise or create disturbance
- enter a property without permission
- enter a bedroom unless the occupants are awake and dressed
- ask for personal favours
- ask for food (you may accept food only when it is given voluntarily)
- disturb furniture
- open cupboards
- break anything.

Use of sucking tube to collect indoor-resting mosquitos

You have been instructed in the use of the sucking tube and practised with it in the laboratory. The skills you have learnt can now be applied in the field.

In any village about 10 houses should normally be examined in order to provide a representative sample. The selection of houses for routine collecting is a complex statistical procedure that should be carried out by a competent person. In the case of a single visit, the houses selected should be scattered throughout the village. It is often advantageous to select the poorest and worst-ventilated houses because they usually contain the largest numbers of mosquitos. Houses on the fringe of a village or near known breeding sites will often yield more day-resting mosquitos.

Mosquitos caught alive in houses may be kept for 24 hours. This will allow you to check the 24-hour mortality rate among mosquitos collected from sprayed houses and provide an indication of the care exercised when collections are made in unprotected houses.

The collection of mosquitos in a house normally takes place early in the morning and should be done after the occupants are up and dressed. Examine the whole house or, if it is too large, spend up to 15 minutes searching in each room. Pay special attention to those parts of the house most likely to yield vector mosquitos, including rooms in which people slept the previous night, the washing room, and any other areas suggested by experience. Try to select rooms with few external openings. Mosquito catches tend to be highest on mornings after heavy rain or when the humidity level is high.

Use of sucking tube

With the aid of the torch, look for mosquitos on walls, ceilings or the roof. Search systematically, starting from the door and moving clockwise around the inside of the house. Look for specimens on wall hangings and curtains, behind and under furniture, and inside large pots and jars.

No more than five mosquitos should be collected in the sucking tube before you transfer them to a paper cup.

Normal resting places of mosquitos in houses

While collecting, observe the resting places normally chosen by mosquitos. If you record in your notebook what you see, including the numbers of the insects on the upper, middle or lower parts of walls, you can determine the proportions resting in each situation.

Labelling paper cups and making records of collections

During hand collecting you will need to transfer mosquitos to paper cups. You should use a separate cup for each house. The cups must be clearly labelled in pencil with at least the following essential information:

- location
- date and time of collection
- time spent on collecting (minutes)
- house number or householder's name
- type of structure (house, animal shelter, store, etc.)
- whether sprayed, and if so, when
- your name.

Keep a separate record in your notebook of each collection you make.

Preparation of collections and transport to the laboratory

The paper cups should be prepared for transportation as previously described. A number of test-tubes with specimens from a single house can be held together with a rubber band and transported in an insulated picnic box or other container.

LEARNING UNIT 7

Spray sheet collection of mosquitos

Learning objectives

By the end of this Unit you should be able to:

- select a house and, with the permission of the householder, identify a suitable room for spray sheet collection of mosquitos
- prepare the selected room for spray sheet collection
- carry out space spraying with a pyrethrin solution
- collect all the mosquitos that have fallen
- label containers accurately and completely, record results, and transport mosquitos to the laboratory.

Spray sheet collection involves the use of a pyrethrin space spray to knock down mosquitos resting inside a house and collecting them on white sheets spread on all flat surfaces. The mosquitos are transported to a laboratory for processing.

The hand collection of mosquitos that you carried out in Learning Unit 6 is unlikely to result in your obtaining all the mosquitos resting in a house, for the following reasons:

- many mosquitos may be hidden from sight, particularly in houses with thatched roofs;
- roofs, ceilings and the upper parts of walls may be too high for you to reach;
- you may not have sufficient time available to collect all the mosquitos.

The efficiency of hand collecting also varies with the absolute density of mosquitos. It is therefore unwise to use data from hand collections for mathematical calculations.

Using the sheet collection method, it should be possible to collect practically all the mosquitos from a well-closed room sprayed with a fine mist of 0.2–0.3% pyrethrin solution. Even in a less well-closed room you will be able to collect a high proportion of the mosquitos resting there.

This method of collection allows quantitative studies to be undertaken, including measurement of:

- indoor resting density (the number of mosquitos resting indoors during the day)
- seasonal changes in indoor resting density

- the number of mosquitos remaining in a particular room after a hand collection.

On arrival in the laboratory, the mosquitos are examined and dissected to determine:

- their blood digestion/egg development stages
- their ages
- the proportion having sporozoites in their salivary glands
- the proportion that have fed on human blood.

Although spray sheet collecting was usually widely accepted in the past, you may encounter some resistance from householders. The physical appearance of the collecting sheets deteriorates markedly after they have been used only once or twice and some people may be reluctant to have them in their houses, especially in areas where housing standards are relatively sophisticated.

Essential equipment

To carry out pyrethrin spray sheet collection you will need the following equipment: white floor sheets; hand sprayers; a hand lens; pyrethrin solution; small Petri dishes; pill boxes, paper cups or metal boxes; hand lens; forceps; a container, preferably a picnic box, for transporting mosquitos to the laboratory; cotton wool; filter paper; a torch (with spare bulb and batteries); a pencil; and a notebook.

The white sheets (sizes 2 m × 2 m and 2 m × 1 m) can be made of cotton; it is often useful to have one or two larger sheets. The number of sheets required depends on the average size of the rooms.

The hand sprayers should be of the double-action type with an air valve (see Fig. 10). Pyrethrin solution, at a concentration of 0.2–0.3% in kerosene, is normally available ready-mixed from your store in quantities sufficient for several weeks' work. If new stocks are required you should prepare them only under the guidance and supervision of a person familiar with the methods used.

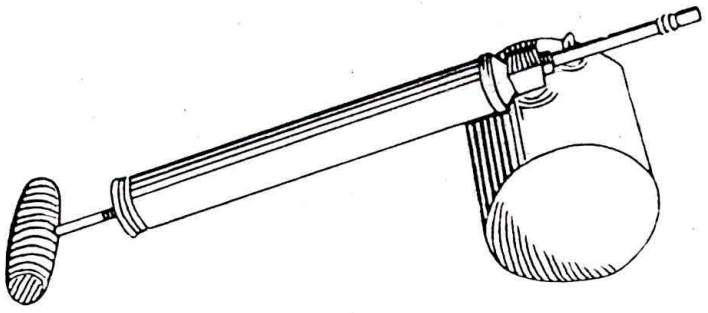


Fig. 10. **Hand sprayer**

If there is likely to be a delay between collection and delivery to the laboratory, wide-mouthed vacuum flasks or an insulated picnic box should be used so that the Petri dishes or other containers for the mosquitos can be kept cool over ice bags.

Selection of rooms for spray sheet collection

Collections are normally made in the morning after the occupants of houses have risen and dressed. Houses will already have been selected if the work is to be done in a locality where regular observations are made; otherwise, selection is a responsibility of the supervisor. In principle, the houses chosen should be distributed throughout the locality.

Permission is required from householders to make collections in their houses.

It is normal for the work to be performed by a team of three or four people so that collections can be made in eight to ten rooms in each locality.

Preparation of rooms for spray sheet collection

Ensuring that you disturb any resting mosquitos as little as possible, prepare a room for spraying as follows:

- Remove all animals and birds.
- Remove or cover all food.
- Remove all small items of furniture.
- Cover all openings and eaves with cloth or mosquito netting.

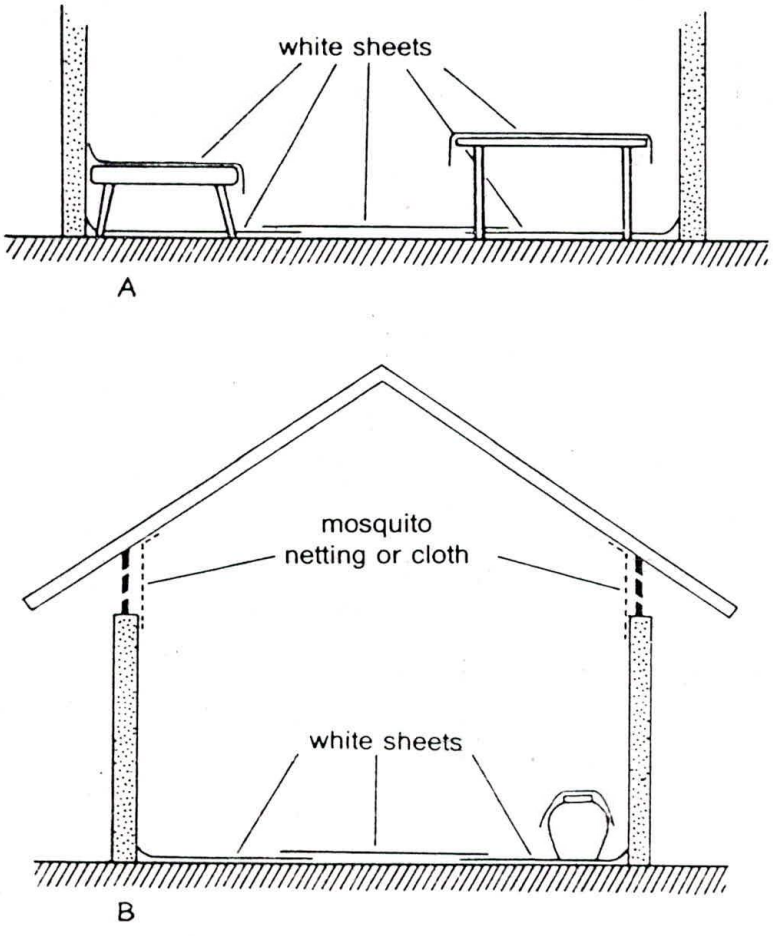


Fig. 11. **Preparation of a room for pyrethrin spraying**

- Spread the white sheets so that they completely cover the floor and all flat surfaces of the remaining furniture. Sheets should also be spread under tables, beds and other places where mosquitos may hide. Sheets placed on furniture should *not* hang down to the floor because this would prevent the spray from reaching mosquitos under the furniture (see Fig. 11).
- Close all windows and doors.

Carrying out space spraying

One of the team members should walk round the outside of the room and spray in open spaces or holes in the walls and eaves. The same person or another member of the team should then enter the room, close the door and, moving in a clockwise direction, apply spray towards the ceiling until the room is filled with a fine mist. The time needed for spraying obviously depends on the size of the room. The operator should leave the room quickly and make sure that the door remains closed for at least 10 minutes. Great care should be taken to avoid treading on any mosquitos that fall early. Many workers remove their footwear to avoid contamination of the white sheets.

Collection of mosquitos from sheets

Two methods can be used to collect mosquitos from the sheets following space spraying with pyrethrin. Each involves picking up the mosquitos with forceps and placing them in a container for transport to the laboratory. To prevent mosquitos from drying out if they are to be used for dissection, there should be a layer of damp cotton wool covered by filter paper in the container.

Method 1

Open the room. Move gradually through the room, starting from the doorway. With the aid of a torch, collect the mosquitos from the sheets; leave the sheets in position.

Method 2

Open the room. Starting from the doorway, pick up the sheets one at a time by their corners. Carry the sheets outside. Collect and examine the mosquitos outside in daylight.

If it is raining or windy, the first method should be used. Collect up and fold the sheets; ensure that you leave no dust or debris behind.

Labelling containers and transporting them to the laboratory

A separate container should be used for the mosquitos collected in each room. The containers should be labelled with all relevant data, including:

- location
- date and time of collection
- house number or householder's name
- type of structure (house, animal shelter, store, etc.)
- number of people and/or animals in the room during the previous night

- whether the room has been sprayed previously and, if so, the date of spraying
- name of collector or supervisor.

This information must be written in pencil directly on paper cups or on slips of paper which are placed inside other types of container.

If the mosquitos that have been collected are to be transported rapidly to the laboratory, the containers can be placed in a box for the journey. However, if there is to be any delay before transport, a wide-mouthed vacuum flask holding ice should be used to keep the containers cool so that the mosquitos remain in a suitable condition for dissection; alternatively, an insulated picnic box with ice packs may be used if one is available.

LEARNING UNIT 8

Outdoor collection of mosquitos

Learning objectives

By the end of this Unit you should be able to:

- describe the sites and methods used to collect resting adult anopheline mosquitos from natural outdoor locations
- collect outdoor-resting mosquitos using a sucking tube
- collect outdoor-resting mosquitos using a drop net
- collect outdoor-resting mosquitos using a hand net
- construct artificial shelters and collect anopheline mosquitos from them
- label paper cups completely and accurately, maintain records of collections, and transport mosquitos to the laboratory.

Some mosquito species enter houses at night to bite and rest indoors during the day. Other species do not enter buildings but bite outside and then rest in the following kinds of outdoor location:

- on vegetation;
- on solid surfaces in sheltered places, such as the banks of streams and ditches, holes in rocks, culverts, cracks in stone walls, caves, animal burrows, on the trunks or stems of larger vegetation such as banana trees, and in old termite mounds.

Outdoor collecting is performed in either the natural resting places described above or in shelters specially constructed for the purpose. Artificial shelters have the advantage of providing concentrated sites for collections and more representative samples that can be used for quantitative work.

Data from outdoor collections are important in evaluating the impact of any anti-vector measures, and provide information about:

- the species that habitually rest outdoors
- the relative numbers of mosquitos resting outdoors
- seasonal changes in outdoor resting habits
- any alterations in the relative numbers of mosquitos resting outdoors following the application of insecticides in houses and other buildings.

Smears made on filter paper from blood-fed specimens may be subjected to precipitin tests to determine the host preferences of mosquitos.

Essential equipment

The equipment required for outdoor collecting consists of: a sucking tube; a torch (with spare bulb and batteries); a hand net; a drop net; paper cups with net covers; cotton wool; an insulated picnic box; a towel; newspapers for use as packing material; a pencil; and a notebook.

Since the preparation or construction of artificial shelters will be undertaken during field practice, you also require: a barrel; two spades; a pickaxe; and an axe or machete.

Choice of method for outdoor collecting

The choice of method for outdoor collecting depends partly on the behaviour of the malaria vector; that is, whether it prefers to rest on vegetation or on solid surfaces.

The three common methods used to collect mosquitos resting on vegetation involve the use of a sucking tube and torch, a hand net, and a drop net.

Anopheline species that normally rest on solid surfaces are collected with the aid of a sucking tube and torch from natural or artificial shelters. Both patience and skill are essential in collecting from natural shelters, since the population density of mosquitos is usually much lower than in artificial shelters and the resting surfaces are irregular.

Collecting mosquitos outdoors with a sucking tube

Direct collection from vegetation using a sucking tube (and transferring captured mosquitos to a paper cup) usually takes a considerable time and may enable you to find only a few mosquitos. Collections from both natural and artificial shelters are also made using a sucking tube and torch. Well-placed shelters normally yield more mosquitos than natural environments.

There is an important difference between these two kinds of collecting in respect of the information that should be recorded:

- After searches in vegetation you should record the number of collections and the total time spent searching.
- After searches in artificial shelters you should record the number of shelters examined and the time spent in each.

Collecting mosquitos outdoors using a hand net

A hand net (or sweep net) like that illustrated in Fig. 12 is used to collect mosquitos resting on vegetation. The correct method of use is to move the hand net swiftly over the tops of tall grasses or close to the ground around bushes.

Make sure that you record the number of collections and the total time spent collecting.

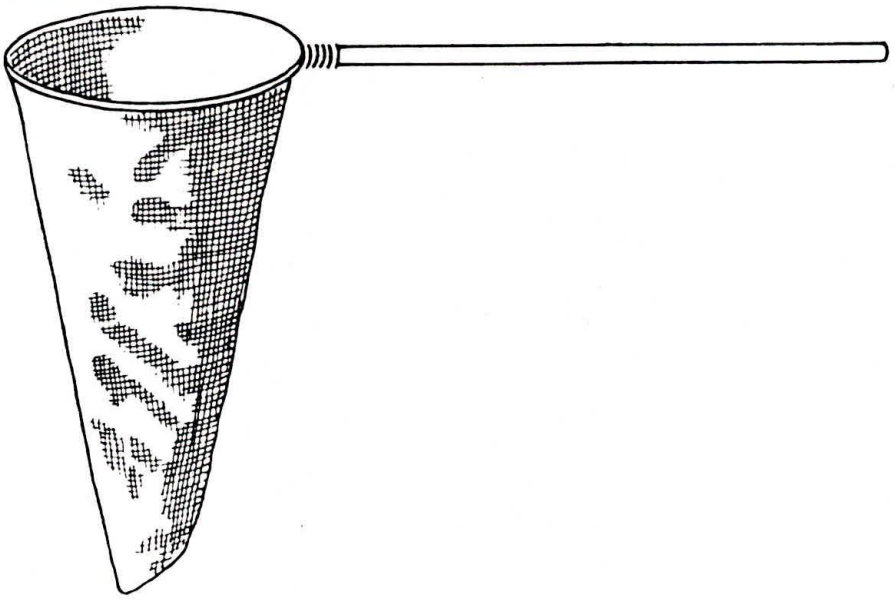


Fig. 12 **Hand net**

Collecting mosquitos outdoors using a drop net

A drop net (Fig. 13) consists of a light, collapsible wooden frame measuring about 2 m × 2 m × 2 m, with its top and sides covered by a mosquito net.

The drop net is placed over grass or low vegetation. Any mosquitos beneath it are disturbed with a stick so that they fly up and rest on the net. The mosquitos are collected from inside the net with a sucking tube and transferred to a paper cup.

Record the number of times the drop net is positioned for collecting; this indicates the area that has been searched.

Collecting mosquitos from artificial outdoor shelters

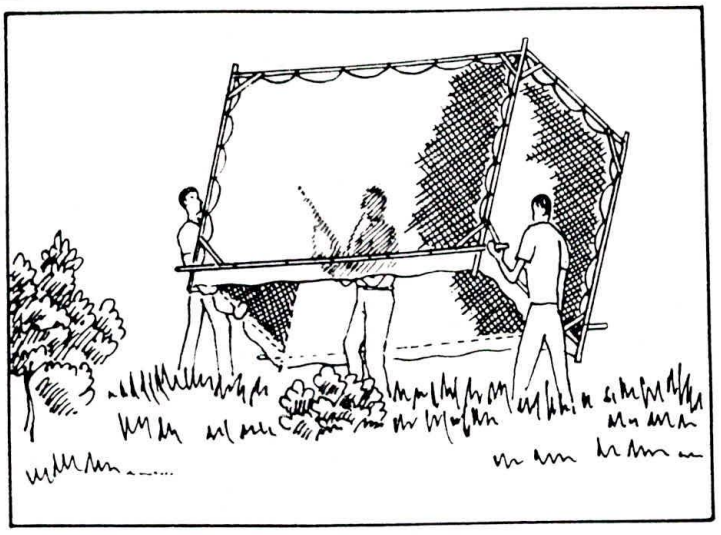
If the anopheline vectors prefer to rest on solid surfaces outdoors, it is preferable to build shelters to attract them. Artificial shelters are easier to search than natural resting places; they may consist of large barrels or boxes, perhaps set into river banks, or they may be pits dug in the ground (see Fig. 14). In general, however, artificial shelters of this type are used only in locations where regular mosquito collections can be made and should be sited where they are unlikely to be flooded in the wet season.

Collections from outdoor shelters are made using a sucking tube and a torch. Before entering any natural or artificial shelter, it is important to make sure that it contains no snakes or other small wild animals.

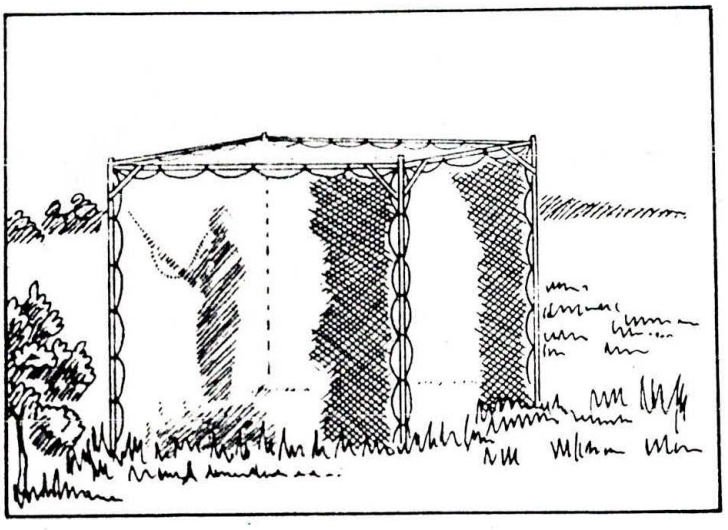
The number of shelters that have been searched must be recorded.

Labelling and recording collections

All paper cup containers must be labelled in pencil with the following information:



Net being lowered



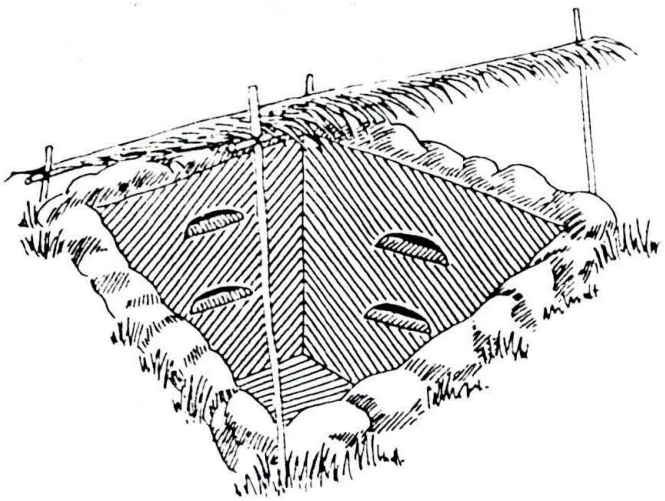
Net in place

Fig. 13. **Drop net for collecting mosquitos from grass**

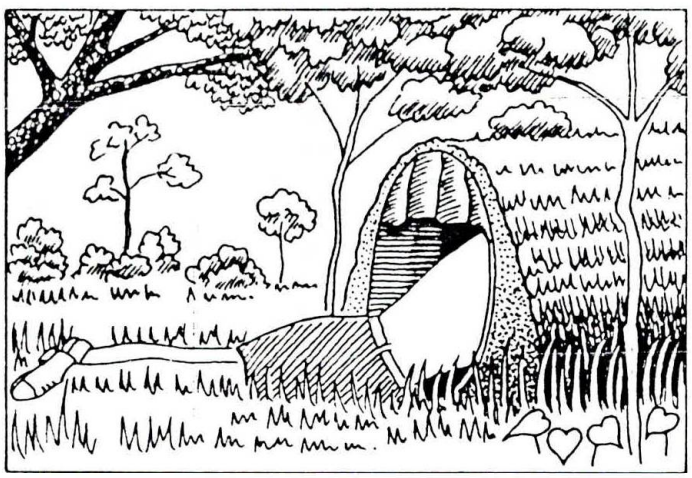
- location
- method of collection
- date and time of collection
- whether the nearest village was treated with insecticide, and when
- the collector's name.

The data derived from outdoor collections are used to calculate the following indices:

- number of mosquitos collected per person-hour, based on outdoor collection with a sucking tube and/or a hand net;
- number of mosquitos collected per unit area, based on outdoor collection with a drop net;
- number of mosquitos collected per artificial shelter, based on outdoor collection from such shelters.



Pit shelter with roof (top view)



Barrel as resting place covered with earth and branches

Fig. 14. **Artificial outdoor shelters**

LEARNING UNIT 9

Direct catches of mosquitos from bait

Learning objectives

By the end of this Unit you should be able to:

- describe when, where and how to make direct collections of mosquitos from human bait
- perform direct-biting collections from human bait
- describe when, where and how to make direct-biting collections of mosquitos from animal bait
- perform direct-biting collections of mosquitos from animals
- maintain, and completely and accurately label and record, collections from the field
- prepare collections for transport to the laboratory.

Female mosquitos are attracted to humans and/or animals to obtain blood meals. The number of vectors biting humans is therefore a major determinant of malaria transmission, and it is important to know:

- which anopheline mosquitos bite humans
- which of the anophelines that bite humans are vectors of malaria
- how often a person is bitten by a vector
- the time of day when vectors bite
- the peak biting time
- whether the vectors bite indoors or outdoors
- the seasonal variations in the numbers of mosquitos biting humans.

Following the start of a malaria control operation it is also necessary to determine whether there are changes in:

- the anopheline species biting humans
- the numbers of vectors biting humans
- the numbers of vectors biting humans indoors compared with the numbers doing so outdoors.

Many vectors bite both humans and animals. Collections should therefore be made on animals to discover whether the vector:

- bites only humans
- prefers humans but also bites animals
- prefers animals but occasionally bites humans.

Essential equipment

In order to capture mosquitos as they are biting you need the following equipment: a sucking tube; a test-tube; a torch (with spare bulb and batteries); paper cups with net covers; rope (for tethering the animal bait); long metal or wooden pegs (to which to tie the tether); hammer; cotton wool; towels; an insulated picnic box with packing material; a pencil; and a notebook.

General rules

Certain important rules should be observed while carrying out this type of collection:

- Do not smoke while collecting.
- Change the people being used as human bait hourly, so as to minimize possible differences in their attractiveness to mosquitos.
- Do not use any oil or ointment that might act as a mosquito repellent.

Siting of bait

Human bait indoors

If possible, select a house in that area of the village with the greatest number of cases of malaria. The house should be typical of the locality.

A house with more than one room is preferred, allowing the usual residents to sleep in one room while another is used by the collector(s). If possible, the room used for collecting should be one in which the residents would normally sleep.

Unless it is raining, collections should not be made on an open verandah, which is considered to be neither inside nor outside the house.

Human bait outdoors

The person acting as human bait for outdoor collecting should be positioned in the general area of the house or room selected for indoor collecting. The site selected should be an area of the village where local people normally sit during the evening.

Animal bait

It is usual for collections from animals to be made outdoors, even in areas where livestock is normally stabled. It is difficult and dangerous to collect directly from a large animal in an enclosed space, especially when other animals are present.

The animal chosen should be stationed a short distance from other animals. It can then be assumed that blood-fed mosquitos resting on nearby vegetation have taken blood from the bait animal, and these can also be collected.

Efficiency of direct collecting

Collections taken directly from human bait using sucking tubes normally reflect quite accurately the number of mosquitos biting. Collections made using trap nets with human bait generally yield fewer mosquitos and less representative samples.

Direct collecting from large animals is very difficult to carry out efficiently and only a small proportion of the mosquitos biting will be obtained. Much greater numbers of mosquitos are usually captured by using a trap net baited with an animal.

When and where to make direct catches from human bait

Direct collecting of biting mosquitos is performed during the night because this is when most malaria vectors take blood meals. The collections are often made during the entire period from dusk to dawn. However, this should be done only when absolutely necessary, and then by two teams of collectors, each working half the night. If the peak biting time is known, collecting can be confined to this period. Peak biting times can be deduced early in a programme from the results of several all-night collections, allowing subsequent regular collections to be made over a period of only three to four hours. Thus, both indoor and outdoor collecting from human bait may be conducted to accommodate the normal resting and sleeping habits of the local people. Remember that night-time habits may vary seasonally.

The times and locations for night collecting will be selected by the supervisor.

It is important that you do not smoke during collections and that you keep as still as possible when collecting mosquitos from your own body.

Direct collecting of biting mosquitos from human bait

The collection of mosquitos from your own body is a common way of obtaining biting specimens, especially of *Anopheles*. The technique is as follows (see Fig. 15):

- Select a quiet place either inside or outside a house as instructed by a supervisor.

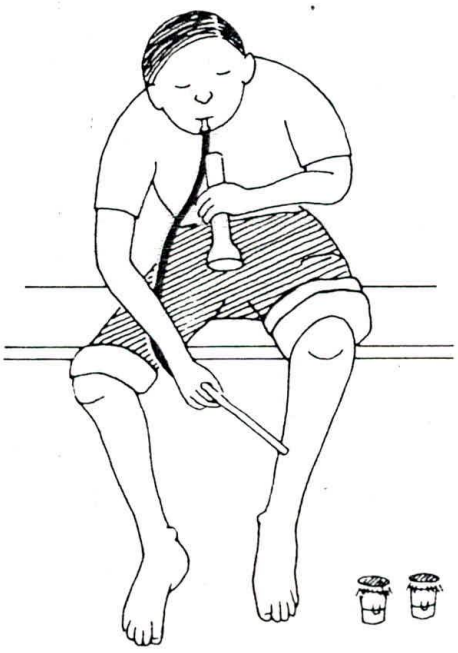


Fig. 15. **Collecting from your own body**

- Adjust your clothing so that your legs are exposed as far as your knees. Then sit quietly.
- When you feel a bite, quickly turn on your torch to find out whether the mosquito is anopheline or culicine.
- If the mosquito is anopheline, or if you are unsure, collect it with your sucking tube and transfer it to a container. Use one cup for each hour of collecting.
- Do not smoke or move around.

Alternatively, one person can serve as bait and another as collector (see Fig. 16). In this case, the technique is as follows:

- The person acting as bait sits or lies in a quiet place, inside or outside the house as appropriate, with his or her clothing adjusted to expose as much skin as is acceptable.
- Using a torch and a sucking tube or test-tube, the collector checks for and collects biting anophelines every two or three minutes.
- The mosquitos are transferred to a paper cup. One cup is used for each hour of collecting.

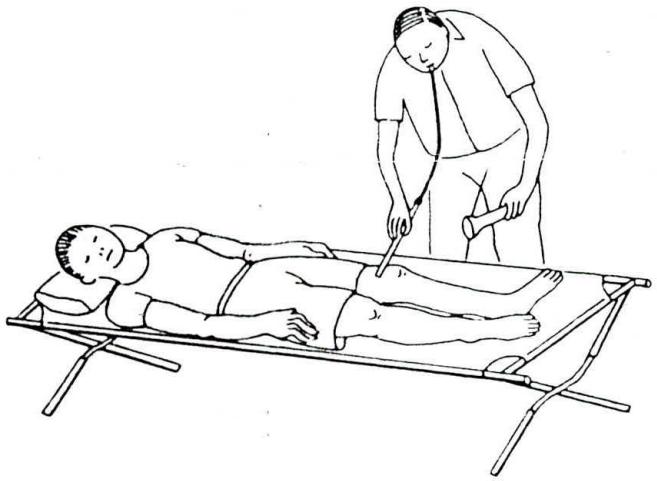


Fig. 16. **Collecting from another person's body**

Ethical considerations

In the past it was considered acceptable to allow mosquitos to bite a person engaged in night collecting. However, for ethical reasons it is now desirable to examine whether health workers should be routinely exposed to the risk of contracting malaria. In fact, it is not necessary to permit mosquitos to feed: they can be collected as soon as they settle on the skin, since it can be safely assumed that biting would normally follow. Landing rates should therefore be measured instead of biting rates.

Nevertheless, those involved in collecting will continue to be bitten; since mosquito repellents cannot be used, this is unavoidable. It is, of course, understood that both collectors and people serving as bait should take an appropriate drug for prophylaxis of malaria.

When trap nets are used, the risk to collectors can be minimized by using protective inner nets (see Fig. 17), although this reduces the efficiency of collection.

When and where to collect from animal bait

Many malaria vectors are attracted in large numbers to animals, and collecting mosquitos on or around an animal can therefore be highly productive.

Collecting from animal bait is normally carried out in the same location and at the same time as collecting from human bait. The exact period of collection is chosen by the supervisor.

Before sunset, select a tame animal from the village, usually a cow or water buffalo. Negotiate with the owner and explain how the animal is going to be used. The collecting site should be near the place where the animal usually passes the night. Then proceed as follows:

- Before sunset, tie the animal securely using a short tether attached to wooden or metal pegs driven firmly into the ground.
- At sunset begin collecting mosquitos by means of a torch and sucking tube.
- Examine the animal every two to three minutes and collect all the anopheline mosquitos you find.
- Keep each hour's collection in a separate paper cup.

Labelling, recording and preserving hourly collections

The paper cups containing the hourly collections must be very clearly labelled to ensure that no confusion arises. The information to be recorded on the cups is as follows:

- location
- date
- whether and when the location was last sprayed
- type of bait (human or animal)
- site of collection (indoors or outdoors)
- hour of collection (e.g., 18 h to 19 h)
- the collector's name.

Keep the paper cups in an insulated picnic box which, when closed, will not allow ants to get in. The humidity in the cups should be maintained by means of damp cotton wool pads.

Transportation to laboratory

Collections should be transported to the laboratory in the picnic box. Take all the necessary precautions you have already learnt about.

LEARNING UNIT 10

Collecting mosquitos in baited trap nets

Learning objectives

By the end of this Unit you should be able to:

- explain when collections should be made from human-baited trap nets
- collect mosquitos in a human-baited trap net
- explain where collections from animal-baited trap nets should be made
- collect mosquitos in an animal-baited trap net
- maintain, and completely and accurately label and record, trap net collections
- prepare collections for transportation to the laboratory.

Collecting mosquitos from human and animal baits necessarily involves a large number of workers if good results are to be obtained. Methods have therefore been developed that use trap nets with either human or animal bait. These require fewer collections and therefore allow the workers periods of sleep during a night's work. These alternative methods also provide valuable information about the numbers of mosquitos biting humans and animals, and are thus often used in place of direct collections.

Animal-baited trap nets generally produce more mosquitos than can be collected by direct capture from animals; the opposite, however, is true for human-baited trap nets. For this reason, standard night collecting from bait usually involves direct collection from humans indoors and outdoors, and collection from animal-baited trap nets outdoors.

In this Unit you will learn how to use trap nets; the purposes of collection by this method are the same as those of direct collecting, noted earlier.

Essential equipment

You will require the following equipment in order to set up trap nets for two human baits (indoors and outdoors) and one animal bait (outdoors): a sucking tube; a torch (with spare bulb and batteries); paper cups with net covers; cotton wool; towels; an insulated picnic box; a pencil; a notebook; an alarm clock; two camp beds; two small mosquito nets with frames to fit the camp beds; two trap nets for human bait; one trap net for animal bait; long metal or wooden pegs and a rope (for tethering the animal); hammer; pegs and string (for securing trap nets); and a needle and thread (for repairing trap nets).

General rules

The following important rules should be observed:

- Do not smoke while collecting.
- People acting as bait indoors should change with those acting as outdoor bait on alternate collecting nights; this allows for differences in attractiveness to mosquitos.
- Keep collections separate according to bait, site and collecting period, and label them correctly.

Collection sites

Two trap nets will be set up in positions selected by the supervisor, one in a sleeping room and the second outdoors at a site where people habitually sit during the evening or sleep.

Collecting by means of human-baited trap nets

A human-baited trap net is set up as shown in Fig. 17 on a site either inside or outside a house. The procedure is as follows:

- Set up a folding camp-bed.
- Put up the inner net around the bed to protect the person acting as bait.
- Erect the outer net by securely tying it to poles or branches.
- Stretch the bottom of the outer net tightly and tie it to pegs in the ground, leaving 15–20 cm between the ground and the lower edge of the net.
- At sunset, get into the trap and lie on the bed or, if someone else is acting as the bait, ask him or her to do so.
- Set the alarm clock to ring after one hour during a season of high mosquito density, two hours in a moderate-density season, or three hours in a low-density season. The collecting period should not exceed ten minutes.
- When the alarm rings, collect all the anophelines in the trap net.

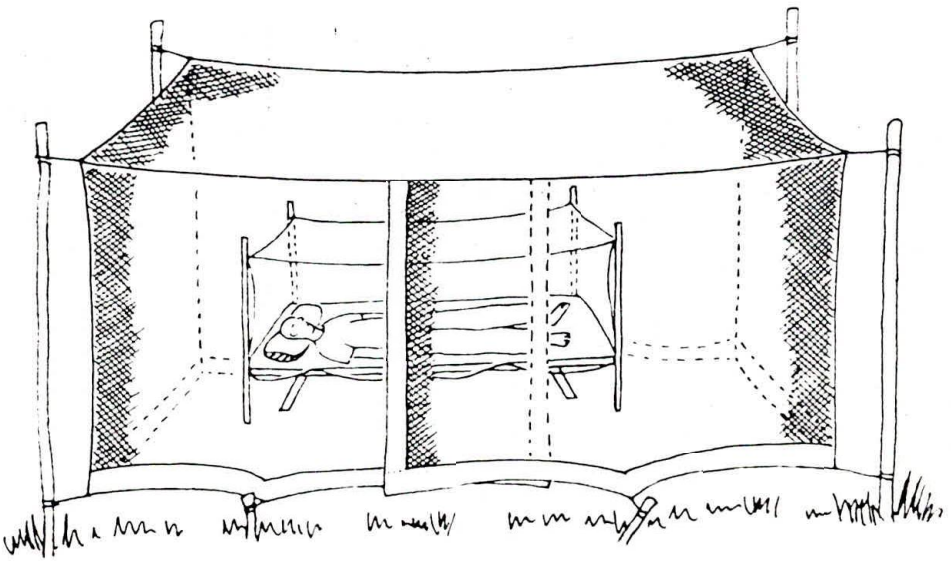


Fig. 17. **Trap net with human as bait**

- Transfer the mosquitos to a container and label it with the date, time, location, and your name. Use one container for each collecting period.
- Get back onto the bed and set the alarm to ring after the same length of time as before.
- Repeat the procedure throughout the night.

If the protective inner bed net is not used, and the mosquitos are thus allowed to feed on the bait, collecting may be done over longer periods, depending on the objective. This is because fully fed mosquitos will be more settled and will rest longer on the sides of the trap net than those that have not fed.

Siting of an animal-baited trap net

An animal-baited trap net is sited close to where the animal is customarily kept overnight.

Animal-baited traps are normally used outdoors only. It is difficult to confine a cow or buffalo in a net in a stable where there are other animals; also, use of this method indoors frequently results in damage to the net.

Collecting by means of animal-baited trap nets

The trap net (see Fig. 18) is similar to that used for collecting mosquitos attracted to human bait. The animal must be securely tethered so that it cannot break free and damage the trap or harm itself. If there is to be repeated collecting at the site, a small enclosure may be built to confine the animal, as shown in Fig. 18.

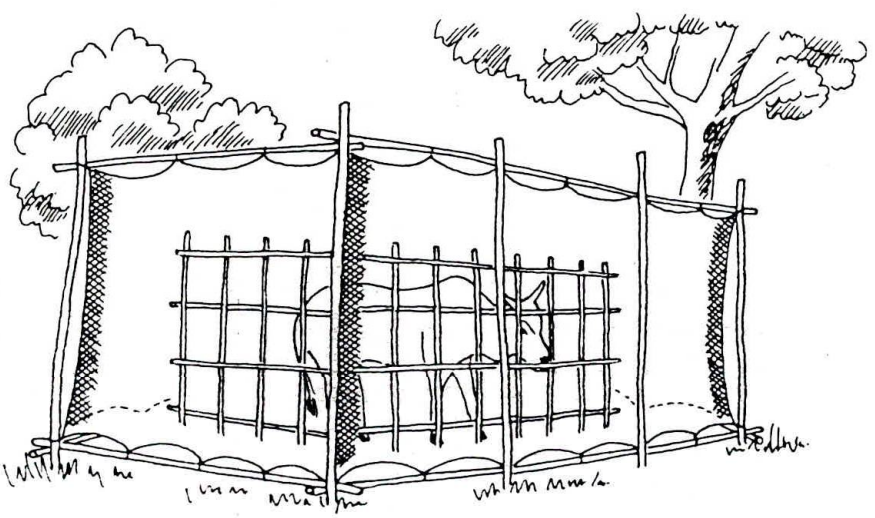


Fig. 18. **Animal-baited trap net**

The animal is placed in the trap at sunset and mosquitos are collected every three hours, for instance at 21 h, 24 h, 03 h, and sunrise. A single collection obtained at dawn produces only about a third of the mosquitos that can be caught if collecting is done every three hours.

Make sure that all containers are labelled correctly. Because of the large numbers of mosquitos obtained when animal baits are used, it may be necessary to use several paper cups during each collection. Do not put more than 50 mosquitos in each cup.

Using animals is a good way to collect large numbers of mosquitos but it does not give accurate information about the times at which biting occurs. This information is best obtained by making hourly collections during the night.

Preserving, labelling, recording and transporting collections

All the procedures described in Learning Unit 9 for direct collecting of biting mosquitos apply equally to collections from baited trap nets.

Note: Nets should be thoroughly dried in the sun, either in the field or on return to the laboratory, before being packed away.

LEARNING UNIT 11

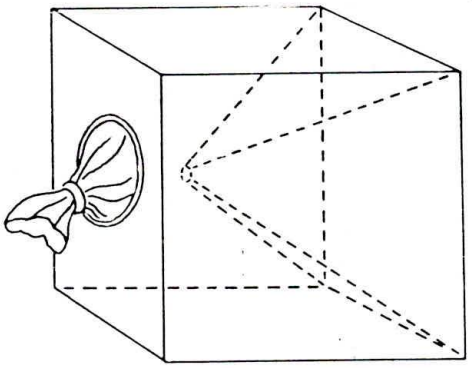
Collecting mosquitos from exit traps

Learning objectives

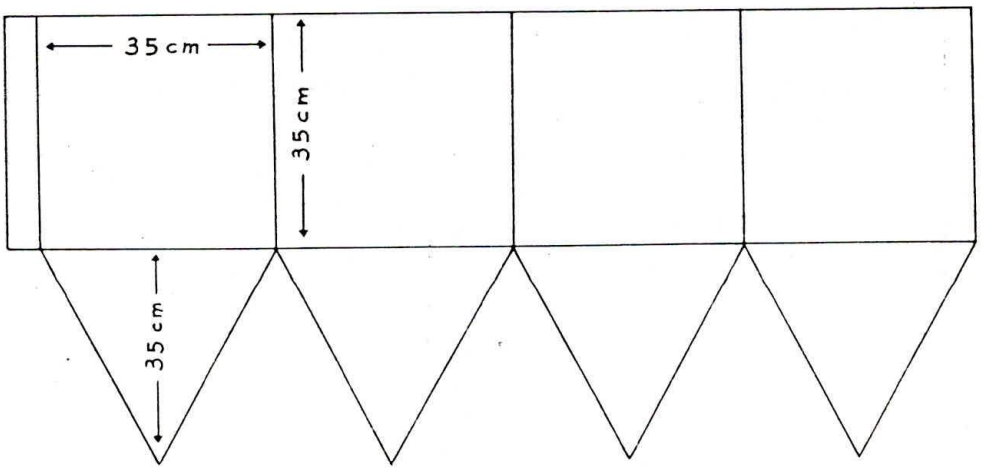
By the end of this Unit you should be able to:

- fit exit traps to houses and other buildings
- describe the precautions necessary for the correct operation of exit traps
- collect mosquitos from exit traps at appropriate times
- label and prepare specimens for transport to the laboratory.

Exit traps (see Fig. 19), which can be fitted to houses and animal shelters, are normally used only for certain special purposes, such as observing the daily



A. Completed trap



B. Pattern for cutting mosquito netting, excluding sleeve side

Fig. 19. **Exit trap**

movements of mosquitos into and out of houses, and distinguishing between the mosquito species that both bite and rest indoors and those that bite indoors but rest outdoors. Studies carried out before and after indoor spraying can also be used to assess the following:

- the effects of insecticides on the normal movement and feeding habits of mosquitos;
- the residual effects of insecticides as indicated by the numbers of dead mosquitos collected and by the 24-hour mortality rate of mosquitos found alive in the traps.

Exit traps are suitable for fitting only to rooms that are well sealed and that have few exit points for mosquitos (see Fig. 20). Collecting by this means must be done under the strict supervision of an experienced entomologist.

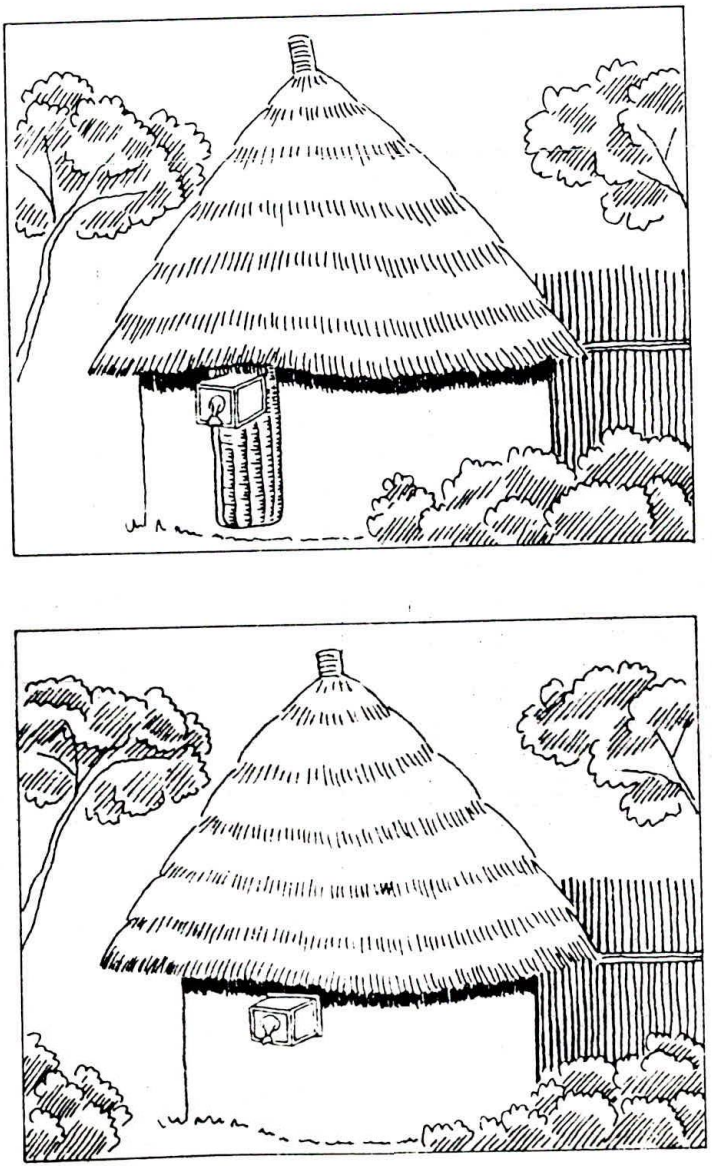


Fig. 20. **Exit traps fitted to door and window**

Essential equipment

Since the type and positioning of exit traps will vary according to the design of houses and animal shelters in particular areas, it is not possible to list the materials necessary for fixing traps. However, the other equipment you will need is as follows: a sucking tube; paper cups with net covers; towels; an insulated picnic box; dark cloth or netting to block openings in rooms; and the exit traps themselves.

Fitting exit traps

The selection of houses and/or animal shelters on which to fit exit traps is important. Those with few openings are the most suitable since efforts must be made to cover or block with dark cloth all openings other than the windows or doors to which exit traps are to be fitted. The mosquitos that enter an exit trap are attracted to the faint light that comes through the trap opening.

It is also important to fix traps in position well before sunset. Collecting should then cover a period of two to three hours after sunset; this will reduce mortality due to nocturnal climatic factors (wind, rain, cold).

One room, normally a sleeping room, is selected and the trap is fitted to a window. The method of fitting depends on the type of house and the window, and also varies according to whether there will be a single collection or regular collections. For regular collections, a permanent arrangement for fixing the trap can be considered, and any parts of the window not covered by the trap can be covered with dark cloth or hardboard.

The trap is fitted to the window with the collecting sleeve outwards. All other large openings, including eaves, have to be covered with dark cloth. A few small openings must remain to allow the entry of mosquitos.

In areas where houses or huts do not have windows, the exit trap may be fixed to the door. In this event, it is constructed with a light wire frame and attached to a piece of thick dark cloth which is longer and wider than the door-frame. The top and one side of the cloth are attached to the door-frame or wall, while the other side and the bottom remain unattached so that people can still enter or leave the house.

Precautions ensuring correct operation of exit traps

The supervisor will explain to the occupants of the chosen house that people should continue to sleep in rooms with traps and that windows to which traps are fitted must remain open. You must check that these windows have been left open when you go to collect mosquitos.

Where traps are attached to doors you should verify, when making collections, that the doors are open and that the cloth is preventing the exit of mosquitos (i.e. there should be no openings through which mosquitos may escape).

If you believe that the occupants may have used an aerosol spray or pyrethrin coil for mosquito control, inform your supervisor.

LEARNING UNIT 12

Collecting larvae and pupae from breeding sites

Learning objectives

By the end of this Unit you should be able to:

- describe the most likely breeding sites of malaria vectors
- describe the methods of collecting mosquito larvae and pupae and demonstrate skill in using a dipper, a larval net, a well net, and a pipette or spoon
- maintain accurate records of all collections of larvae and pupae
- transport live larvae and pupae collected in the field to the laboratory
- kill and preserve larval and pupal specimens.

Each type of mosquito prefers to lay its eggs in a particular kind of water. Some will lay only in fresh, clear, running water with some shade, others only in brackish water; some may even lay eggs in very small quantities of water, such as that in a discarded tin can or trapped in a hoof-print.

It is most important for you to know the preferred breeding sites of the anopheline mosquitos that transmit malaria, and the densities of larvae and pupae at these sites. The methods by which you will collect larvae and pupae will depend on these variables.

Collecting from all the different types of breeding site in an area will allow you to:

- determine the species present
- determine the preferred breeding sites of each vector species
- determine whether larvae and pupae are present during a control programme directed against adult mosquitos even though these may be rare or uncollectable
- make an assessment of the effectiveness of a programme directed against larvae and pupae.

Essential equipment

The equipment required for the various methods of collecting larvae consists of: a dipper; a larval net; a well net; a spoon; a large tray; a pipette; stoppered specimen tubes (vials); 70% alcohol solution or 2% formalin solution; a pencil; and a notebook.

If live specimens are required for insecticide testing you will also need larger bottles or a wide-mouthed vacuum flask.

Preferred breeding sites of anopheline mosquitos

To identify the preferred breeding sites, it is essential to be systematic and check all possible breeding places, even those that are hard to reach. This enables you to determine the types of site most likely to harbour the larvae of anopheline mosquitos.

Potential breeding sites include:

- small pools, tin cans, hoof-prints, drains and ditches, where the entire surface of the water should be examined;
- brackish water, e.g. at sites where fresh water and salt water mix;
- streams, which should be searched at the edges where there is vegetation and the water moves slowly;
- ponds and lakes, where larvae usually occur in vegetation around the edges but can sometimes be found far from the shore among floating vegetation;
- swamps and marshes, where larvae occur in places similar to those described for ponds and lakes;
- special sites, such as wells and water containers made of cement, where the entire surface of the water should be examined.

Anopheline larvae and pupae are often concentrated in certain parts of large breeding sites. If these locations are known, they can be used regularly for collecting.

Whichever collecting method is used, you must always approach the breeding place cautiously, facing the sun: if the larvae are disturbed by shadows and movement many of them will swim downwards and disappear from view. You will then have to wait quietly for several minutes until they return to the surface of the water before you can collect your samples.

Methods for collecting larvae and pupae

Use of the dipper

Various kinds of dipper are used, including small frying pans, soup ladles and photographic dishes (Fig. 21); it is important to use the right type and size for each breeding place. A white enamelled dipper is preferable, because this allows you to see the larvae most easily.

The method of use (see Fig. 22) is as follows.

- Lower the dipper gently into the water at an angle of about 45°, until one side is just below the surface.
- While dipping, care should be taken not to disturb the larvae and thus cause them to swim downwards. If they are disturbed, wait for a minute or two until they come up to the surface again, and then continue dipping.
- Move along the breeding site, skimming the surface of the water with the dipper.
- Lift the dipper out of the water, making sure that you do not spill the water containing the larvae and pupae.
- Hold the dipper steady until the larvae and pupae rise to the surface of the water.

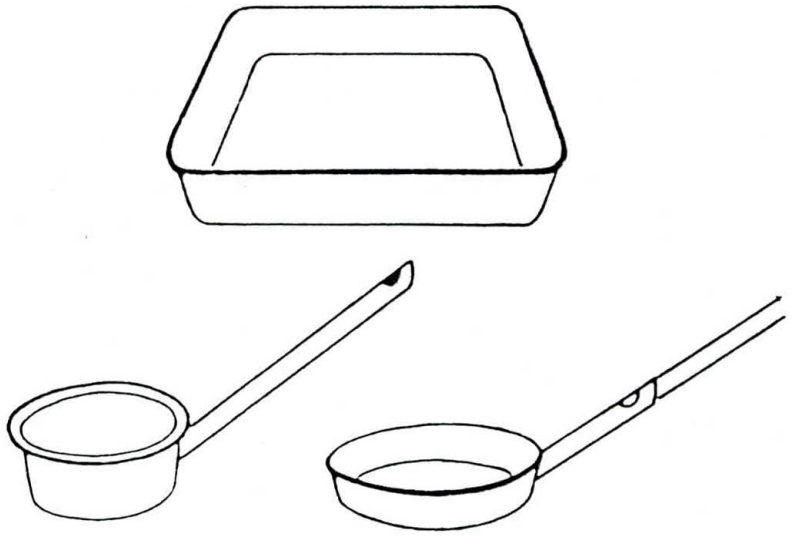


Fig. 21. **Types of larval dipper**

Fig. 22. **Using a larval dipper**

- Collect the larvae and pupae by means of a pipette and transfer them to a bottle or vial.
- Do not throw the residual water back into the breeding place, as this may further disturb the larvae and pupae.

An alternative method is to hold the dipper at an angle of 45° and lower it gently until it is just below the surface so that water flows in with any larvae and pupae that may be present.

Where there is dense, floating vegetation or debris, use the following method:

- Disturb the water, causing the larvae and pupae to sink below the surface.
- Clear away the vegetation or debris with the dipper and wait a few minutes for the larvae and pupae to return to the surface.
- Collect the larvae and pupae with the dipper as described above.

Where there are clumps of vegetation such as tall grasses, press the dipper into the clump and lower it into the water so that the surface water flows in. Then take the dipper out of the water and collect larvae and pupae as indicated above.

Use of the larval net

A larval net for collecting larvae and pupae in ponds and lakes consists of a fine mesh net which has a plastic bottle or tube tied to one end and is mounted on a wooden handle (see Fig. 23). To collect larvae and pupae, sweep the water surface by holding the net at an angle and moving it through the water (see Fig. 24). Larvae and pupae on the water surface will be swept into the net and will collect in the plastic bottle or tube.

Alternatively, a simple net with no attached bottle or tube can be used. After sweeping, the net should be inverted into a bowl of water and its contents dislodged. The water in the bowl is then searched for larvae and pupae, which are picked up and transferred to a bottle or vial by means of a pipette.

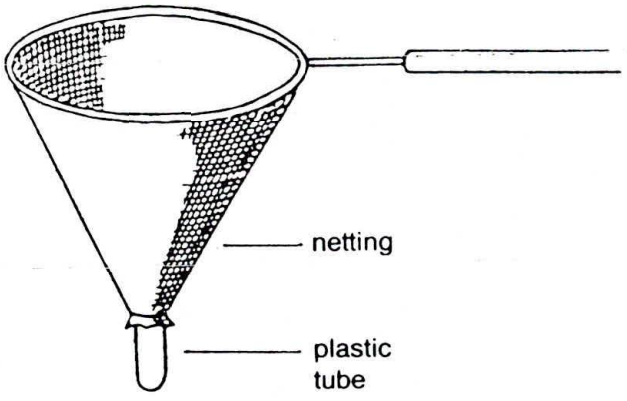


Fig. 23. **Larval net**

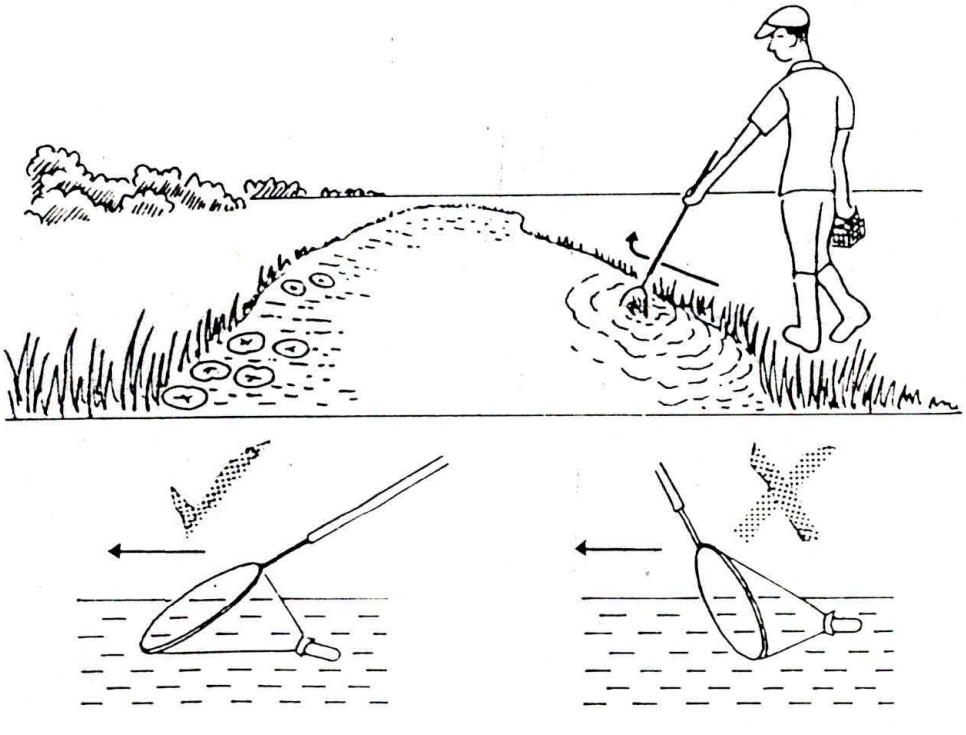


Fig. 24. **Using a larval net**

Use of the well net

The net used for sampling from wells is similar to the larval net but lacks the wooden handle; instead, it is held at an angle by four strings and controlled by a long string or rope (see Fig. 25). The method of use is as follows:

- Lower the net into the well so that the lower side of the net is just under the surface of the water and its opening is at an angle of about 45° (see Fig. 26).
- Move the net right round the side of the well once or twice.
- Withdraw the net and look for larvae and pupae in the bottle or tube at the end of the net.

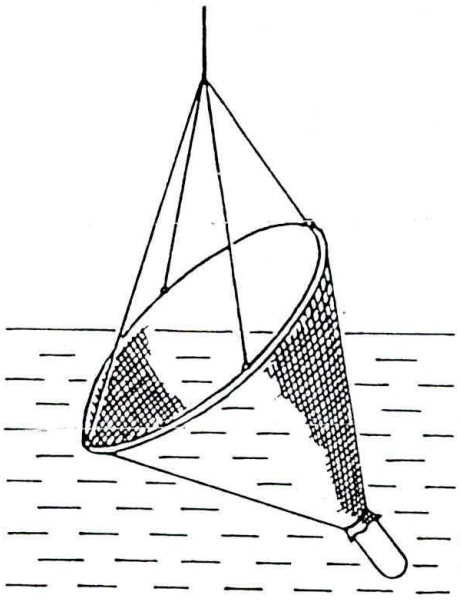


Fig. 25. **Well net**

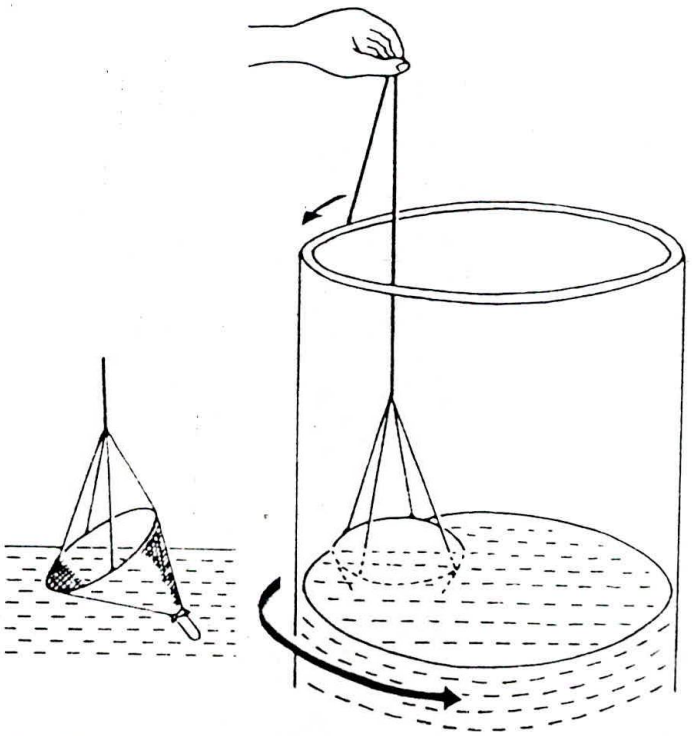


Fig. 26. **Using a well net**

Using a small spoon or pipette

A small spoon or wide-mouth pipette can be used for collecting larvae and pupae from small amounts of water, e.g. in a tin can or trapped in a hoof-print. The method is as follows:

- Stir the water vigorously with a stick to make it muddy.
- Watch for the larvae and pupae to rise to the water surface: they are easily seen against the muddy background.
- Pick up the larvae and pupae with the spoon or pipette.

After collecting by this or any of the above methods, you should transfer the larvae and pupae to specimen bottles or vials.

Maintaining accurate records of collections

Draw a sketch-map to show the positions of the breeding sites relative to villages and settlements. As you collect you should give each breeding place a number in your notebook. Against this number, record the location, type of breeding place, number of dips made or time spent sampling, and the date.

Place all the specimens from a particular breeding place in one bottle or vial and label it with the number recorded in your notebook. The number must be written in pencil on a piece of paper and dropped into the specimen bottle. Do **not** use a ballpoint pen as the ink dissolves in water.

Transporting live larvae and pupae to the laboratory

The larvae and pupae collected must arrive alive and undamaged at the laboratory.

Stopper each bottle or vial tightly so that water cannot spill. Make sure that there is air in the top 1–2 cm so that the larvae and pupae can breathe for a few hours. If a larger air space is left, the water will become agitated during transportation and the specimens will suffer damage, particularly loss of hairs. If the journey to the laboratory takes more than two to three hours, remove the stoppers every two hours to provide the specimens with fresh air.

Pack bottles and vials carefully so that they are not jolted during transportation.

If the larvae are to be used in insecticide susceptibility tests they should be transported in water in a large vacuum flask or other large container.

Killing and preserving larvae and pupae

If the laboratory asks you to provide preserved larvae and pupae:

- Kill them by placing them in warm water i.e., at 60°C.
- Remove the larvae and pupae from the water and put them into specimen tubes containing 70% alcohol.

- Put a label on each tube showing the collection number.
- Close the tubes tightly.

Another commonly used method of killing and preserving larvae and pupae in the field is to add a few drops of 2% formalin solution to vials containing freshly collected larvae or, preferably, to place the larvae in vials containing 2% formalin solution.

LEARNING UNIT 13

Assisting the field supervisor

Learning objectives

By the end of this Unit you should be able to:

- describe what the supervisor expects from you in order to prepare complete and accurate records of your work and maintain equipment in good working order
- describe what kind of information on malaria you are now able to provide to the community.

The field supervisor is responsible for collecting and recording information on project record forms. You can help with this task by ensuring that you carry out all collections correctly and by labelling the paper cups or other containers with all the required information.

It is important that you inform the supervisor of any unusual circumstances that might have affected your collections, such as there being no mosquitos in an exit trap because someone closed the window to which it was fixed, or the use by a householder of an aerosol insecticide or mosquito coil.

Maintenance of equipment

The supervisor is responsible for ensuring that the correct collecting equipment is brought to the field in good condition. The workers collecting mosquitos are individually responsible for the equipment issued to them.

Collectors are usually also responsible for the packing of equipment after use. You must inform the supervisor of any loss or damage to equipment, such as splitting or holing of trap nets and exit traps, or breakage of test-tubes and sucking tubes, so that remedial action can be taken before the next field operation.

Giving information to the community

In a malaria control programme, certain people are usually responsible for providing information on malaria and/or general health education to local communities. Their visits to any particular community do not occur very often, whereas yours will be more frequent. During your visits it is quite likely that people will ask you questions about malaria, mosquitos and the reasons for your work. The information you have received during training should enable you to answer most such questions.

Remember: When answering questions, only give replies that you know are correct. If you cannot answer a question, pass it on to your supervisor.

Explanation of terms used in the manual

adult — The adult (imago) anopheline mosquito is a slender, delicate insect with comparatively long, thin legs. The outer covering of the body is composed of a tough substance called chitin. The body is divided into three distinct parts: head, thorax and abdomen.

anopheline mosquitos — Members of the group of mosquitos having the scientific name *Anopheles*. Only certain species of anopheline mosquito carry human malaria.

Arthropoda — Animals characterized by several jointed legs and a hard outer skeleton, e.g. spiders, ticks, mites, and insects (the group that includes mosquitos).

bioassay — Assessment of the efficacy and persistence of an insecticidal treatment by exposing mosquitos of known susceptibility to a treated surface or area for a standard period of time.

chloroform — A chemical used for immobilizing mosquitos to facilitate their handling. It is toxic and should be used with extreme care.

chloroquine — The drug most commonly used for treating malaria.

climatic data — Information on weather patterns, such as maximum and minimum temperatures, rainfall, and relative humidity.

culicine mosquitos — Most mosquitos that are not anophelines fall into this group. Culicine mosquitos are not vectors of human malaria, but may transmit other diseases (e.g. dengue fever, filariasis, Japanese encephalitis).

drop net — A large net on a rigid frame, which is dropped over vegetation to collect specimens of outdoor-resting mosquitos.

ecological area — A well defined geographical area, for example a tropical rain forest, characterized by certain assemblages of plants and animals (including insects).

eggs — Fertilized female reproductive cells with nutrient material, deposited by female mosquitos and developing in water to produce free-swimming larvae.

endophilic — Tending to rest indoors, whether by day or by night.

exit traps — Devices typically placed over doors and windows of houses or animal shelters to catch mosquitos leaving these buildings.

exophilic — Tending to rest outdoors, whether by day or by night.

feeding habits — Habits determining the times and places of feeding and the sources of blood meals for mosquitos.

gametocyte — These are sexual forms of the malaria parasite that develop within red blood cells. The male gametocytes are called microgametocytes, the female macrogametocytes.

gonotrophic stage	The condition of female mosquitos during blood ingestion and ovarian development.
host preference	The preference of a mosquito for a particular type of host, human or animal. (To be distinguished from simple readiness to feed on a given type of host when no other is available.)
insecticide	A chemical designed to kill insects.
insecticide resistance	The ability of a mosquito to survive contact with an insecticide in quantities that would normally kill a mosquito of the same species.
instars	Stages of insect growth and development. In mosquitos there are four larval instars, each terminating with the shedding of the skin.
larva	The second stage in the life cycle of the mosquito. Larvae develop within the eggs and emerge when the eggs hatch. They swim and eat small organisms on the surface of water.
larval habitat	The type of aquatic environment in which mosquito larvae are typically found.
larval survey	The process of searching for mosquito larvae in a defined area. Larvae are collected and taken to the laboratory for identification.
life cycle	The stages of development through which a plant or animal passes during its life. For mosquitos these stages are: egg, larva, pupa, adult.
malaria parasite	A parasite that lives in the blood of humans and animals. Malaria parasites of humans belong to the group with the scientific name *Plasmodium*.
malaria prophylaxis	Measures taken for protection against malaria, e.g. administration of a drug that prevents a person from becoming infected with the disease.
malaria survey	A study designed to determine the number of people carrying malaria parasites in their blood.
malaria transmission	The process by which a vector mosquito transfers malaria parasites from an infected person to one who is uninfected.
malariologist	A person who has studied and is knowledgeable about malaria and its control.
man/vector contact	The number of times a person is bitten by a vector mosquito, normally expressed as the number of bites per person per night.
mortality rate	The percentage of mosquitos that die within a specified period of time.
mosquito repellent	Any substance producing a negative response in mosquitos, causing them to avoid a close approach (such as alighting on the skin of a host animal or entering a treated room).

oocyst	Fertilized female cell (zygote) after the ookinete penetrates and encysts in the wall of the mosquito stomach. This cell undergoes division to produce sporozoites.
ookinete	Motile (mobile) stage of the malaria parasite resulting from fertilization of the macrogametocyte by microgametocyte(s) in the mosquito gut. After passing through the gut wall, it becomes the oocyst.
operational surveys	Field surveys conducted periodically to evaluate or monitor malaria control methods and their effects.
outbreak of malaria	A sudden increase in the number of people sick with malaria in a particular area (village, town, district).
parasite	A plant or animal that lives on or in, and derives its nutrients from, a host plant or animal.
parasite density	The number of malaria parasites in a specified volume of blood.
parous rate	The proportion of female mosquitos that have laid eggs at least once.
peak biting time	The period during the biting cycle of a given anopheline species when the largest number of females take blood meals.
precipitin tests	Tests in which blood from the stomach of fed mosquitos is analysed to identify the host species.
preferred breeding sites	Sites suitable for egg-laying and satisfactory for all aquatic stages of development.
seasonal prevalence	The number of cases of infection in relation to the unit of population in which they occur (a static measure) at different times of the year.
pupa	The third stage in mosquito development, emerging when the fourth larval instar sheds its skin. Pupae swim but do not feed.
random sampling	The sampling process whereby each unit in the population has an equal chance of being selected.
rate of infection	The percentage of mosquitos carrying malaria parasites. The relationship between the sporozoite rate and the total infection rate is closely dependent on longevity, which may be estimated from these two factors. If an estimation of longevity is required, examination for sporozoites may be supplemented by careful examination for oocysts, and separate rates for oocyst, sporozoite and total infection may be recorded.
red blood cells	Cells in the blood that carry oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs. It is the red blood cells that are attacked by the malaria parasite.

relapse	A renewed manifestation of clinical symptoms and/or parasitaemia associated with malaria infection, separated from the previous manifestation by an interval greater than one reflecting the normal periodicity of paroxysms.
representative sample	A sample that gives an indication of the composition of the whole population.
resistance to drugs	The ability of a parasite to live in the presence of a drug that would normally kill members of the same species.
resting habits	The habits determining the places and times of day or night that mosquitos rest.
salivary glands	The glands that produce the saliva injected when a mosquito bites, which prevents blood from clotting while the mosquito feeds.
sampling	It is usually impossible to collect every individual in a population, such as all the mosquitos in a village. Sampling means taking a small, representative proportion of the total.
species	A fairly well defined, interbreeding group of plants or animals.
species infection rate	The percentage of a species of mosquito carrying malaria parasites.
sporozoite	The final stage of development of *Plasmodium* in the mosquito; this is the infective form of the malaria parasite, occurring either in a mature oocyst before its rupture or in the salivary glands of a mosquito.
surveillance	The monitoring of changes in the numbers of malaria cases over a period of time.
susceptible	The term applied to a vector against which an insecticide is completely effective.
susceptibility test	A test to assess the extent to which a population of a given mosquito species is susceptible to a particular insecticide. Samples of mosquitos are exposed to known concentrations of an insecticide for specified periods.
taxonomic study	Identification of species and their genetic relationship to one another.
transmission	The process whereby the malaria parasite is carried from person to person by mosquitos.
trap nets	Nets used to sample the living mosquito population. By permitting access to a bait but restricting movement away from it, trap nets tend to concentrate female mosquitos near the bait.
vector	An insect or other animal that carries a disease from a plant or animal to another plant or animal of the same species which is free of the disease. Anopheline mosquitos are the vectors of human malaria.

vector density — The number of a given vector species present. It may be expressed in relative terms (e.g. the biting density in relation to the human host) or in absolute terms (e.g. the number present in a room, cattle-shed or artificial shelter).

zygote — The product of the union of a male and a female gamete.

WHO publications of related interest

	Price (Sw. fr.)*
Basic malaria microscopy **Part I: Learner's guide.** 1991 (72 pages)	14.–
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WHO Expert Committee on Malaria Eighteenth report WHO Technical Report Series, No. 735. 1986 (104 pages)	14.–
Malaria control as part of primary health care Report of a WHO Study Group WHO Technical Report Series, No. 712. 1984 (73 pages)	8.–
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Manual on environmental management for mosquito control, with special emphasis on malaria vectors WHO Offset Publication, No. 66. 1982 (283 pages)	22.–
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* Prices in developing countries are 70% of those listed here.