LARVAL SOURCE MANAGEMENT

A supplementary measure for malaria vector control

AN OPERATIONAL MANUAL

World Health Organization

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Abbreviations

ACT	Artemisinin-based Combination Therapy
BR	Briquettes
Bs	Bacillus sphaericus
Bti	Bacillus thuringiensis subsp. israelensis
CI	Confidence Interval
CORPs	Community-Owned Resource Persons
DDT	dichloro-diphenyl-trichloroethane
EC	Emulsifiable Concentrate
EIR	Entomological Inoculation Rate
GIS	Geographical Information System
G	Gram (weight)
GMAP	Global Malaria Action Plan
Ha	Hectare
IGRs	Insect Growth Regulators
IPTp	Intermittent Preventive Treatment in pregnancy
IRS	Indoor Residual Spraying
ITN	Insecticide-treated Mosquito Net
ITU	International Toxic Units
IVM	Integrated Vector Management
L	Litre (volume)
LCP	Larval Control Personnel
LLIN	Long-lasting Insecticidal Net
LSM	Larval Source Management
LV	Low Volume
M	Metre (length)
MDA	Mass Drug Administration
MG	Microgranule
Min	Minute (time)
MIS	Malaria Indicator Survey

MFI	Malaria Free Initiative
MMF	Monomolecular Film
NMCP	National Malaria Control Programme
R0	Basic Reproductive Number
RBM	Roll Back Malaria Partnership
RDT	Rapid Diagnostic Test
SC	Suspension Concentrate
TCU	Ten Cell Unit
ULV	Ultra Low Volume
UMCP	Urban Malaria Control Programme
WG	Water-Dispersible Granule
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme
WP	Wettable Powder
WSP	Water Soluble Pouches

Introduction

Global malaria control efforts have produced remarkable results over the past decade, during which an estimated 1.1 million malaria deaths were averted, with 58% of these lives saved in ten countries with the highest malaria burden in sub-Saharan Africa (*1*). There were an estimated 219 million episodes of malaria (uncertainty range: 154–289 million) and an estimated 660,000 deaths (uncertainty range: 490,000-836-000) due to malaria in 2010. Much of the recent decrease in the global malaria burden has been achieved through the scale-up of vector control interventions, particularly the use of insecticides for treating mosquito nets – long-lasting insecticidal nets (LLINs) and insecticide-treated nets (ITNs) – and other materials, as well as for indoor residual spraying (IRS) (*2*).

In recent years, there have been calls for widespread scale-up of larviciding for malaria vector control in sub-Saharan Africa, although the necessary evidence of impact on malaria transmission is lacking. Notably, the significant progress in malaria control reported in recent years from most parts of the world did not result from the use of larviciding or any other form of larval source management. This situation prompted the World Health Organization to issue, in 2012, a position statement on larviciding (*3*), recommending that: larviciding can be a useful supplement to core interventions but only in some specific locations, where vectors tend to breed in permanent or semi-permanent water bodies that can be readily identified and accessed, i.e. breeding sites which are 'few, fixed and findable', and where the density of the human population to be protected is sufficiently high to justify the necessary resources. Larviciding is therefore potentially suitable as a supplement to core interventions for some clearly delineated habitats, particularly in urban areas, but not in most rural areas of Africa where larval habitats are both numerous and unstable. WHO also recommended that resources for core interventions (LLINs and IRS) should not be diverted for larviciding in such settings.

A number of countries are currently implementing larviciding, and WHO continues to engage with these countries to ensure that larviciding and other forms of larval source management are implemented well and cost-effectively. This operational manual has therefore been prepared to provide guidance on larval source management (LSM) as a supplementary approach to vector control. The manual is intended for use by managers of malaria control programmes, field staff and policy makers. It provides complete step-by-step guidance on the planning, implementation, management and evaluation of LSM programmes, and updates previous WHO technical guides to LSM (*4,5,6,7*).

1. LSM for malaria control

1.1 LSM in malaria vector control

While LLINs and IRS remain the backbone of malaria vector control, as they can be rapidly scaled up across a wide range of ecological and epidemiological settings, larval source management is an additional strategy for malaria control in Africa. Unlike LLINs and IRS, which target the adult mosquito vector, LSM targets the immature, aquatic stages of the mosquito (the larvae and pupae), thereby reducing the abundance of adult vectors. If all potential breeding sites were eliminated or

treated (unlikely for most rural areas of sub-Saharan Africa), it could be expected that LSM would reduce the number of infective bites per person per year (the Entomological Inoculation Rate [EIR]), thereby reducing malaria transmission (*8,9*, and **Annex 1**). In well-defined settings where it is feasible, the elimination of larval habitats can be a cost-effective and long-term solution (*10,11,12*).

What is LSM?

Malaria is transmitted by female mosquitoes of the Anopheles genus (anophelines). The life cycle of the mosquito has four stages: egg, larva, pupa and adult (see **Annex 1**), the first three of which are aquatic.

Larval Source Management (LSM) is the management of aquatic habitats (water bodies) that are potential larval habitats for mosquitoes, in order to prevent the completion of development of the immature stages (*13*). There are four types of LSM:

1. Habitat modification: a permanent alteration to the environment, e.g. land reclamation;
2. Habitat manipulation: a recurrent activity, e.g. flushing of streams;
3. Larviciding: the regular application of biological or chemical insecticides to water bodies;
4. Biological control: the introduction of natural predators into water bodies.

Until the 1950s, LSM was the primary method of malaria control. For example, environmental management was used to control malaria in the early 20th century by the Tennessee Valley Authority (*14,15*) and engineering works were used to control malaria and dengue to protect the workers constructing the Panama Canal (*16*). Larviciding with Paris Green[1] was used to eliminate *Anopheles gambiae* from Brazil by 1940 following its introduction in the late 1920s (*17*), and from Egypt by 1945 following its introduction in the 1940s (*18*). After the introduction of IRS with DDT in the 1950s, and ITNs in the 1990s, LSM was used less commonly in Africa.

Today there is renewed interest in LSM (*19–32*) and its practical application in Africa as a complementary intervention to LLINs and IRS, especially where outdoor biting by malaria vectors is problematic or where there is resistance to the insecticides used for LLINs or IRS (*22,29,33*). Field trials in different eco-epidemiological settings in Africa and Asia (where larval habitats were few, fixed and findable) have shown that larviciding can reduce the density of adult vectors and consequently malaria transmission and morbidity (*21,22,31*). However, in other field trials it has been shown that LSM does not work in every ecosystem, for instance it performed poorly in areas with extensive flooding, where larvicides were applied by ground teams (*23*).

1.2 How effective is LSM?

The evidence on the effectiveness of LSM in controlling malaria is presently being examined in a Cochrane Review (*13*). LSM can be a major logistical and financial undertaking and on-going research into its effectiveness in various environmental and epidemiological settings is therefore required.

Although there are well-documented accounts of successful LSM programmes, there are also numerous examples of LSM failing in situations where the intervention was incorrectly applied or applied in inappropriate ecological settings, resulting in a waste of resources. This manual therefore aims to assist programme managers in deciding where and how LSM should and should not be applied.

[1] *Paris Green* is the common name for copper acetoarsenite, a highly toxic emerald green crystalline powder. It was a popular pesticide from the late 1900s and remained commonly employed on fruit in the USA until the 1970s. For *Anopheles* larval control, a floating dust formulation was used extensively through the 1940s, including in Sardinia, Brazil and Egypt. *Paris Green* has since been replaced by synthetic larvicides with a greater margin of safety.

WHO Interim Position Statement: the role of larviciding for malaria control in sub-Saharan Africa

The WHO Position Statement on larviciding published in 2012 (*3*) should be consulted for full WHO recommendations on larviciding with emphasis on sub-Saharan Africa. In summary, the statement recommends that larviciding will generally be most effective in areas where larval habitats are few, fixed and findable. It specifies that: larviciding measures should normally be used only as a supplement to the core interventions (ITNs or IRS); larviciding should never be seen as a substitute for ITNs or IRS in areas with significant malaria risk; larviciding is most likely to be cost-effective in urban areas where the appropriate conditions are more likely to be present; and in rural settings, larviciding is not recommended unless there are particular circumstances limiting the larval habitats, as well as specific evidence confirming that such measures can reduce the malaria incidence rate in the local setting.

1.3 The role of LSM in Integrated Vector Management

Vector control programmes are now being encouraged to adopt the concept of Integrated Vector Management (IVM) (*35*). The IVM approach seeks to improve the efficacy, cost-effectiveness and sustainability of disease-vector control, through:

- The selection of vector control methods based on knowledge of local vector biology and disease transmission;
- Collaboration between the health sector, other private and public sectors and local communities;
- The use of a range of interventions;
- Rational use of insecticides;
- Good management.

IVM embraces the principle that vector control is not solely the responsibility of the health sector, but can be achieved through coordination of all sectors whose activities potentially affect vector-borne diseases, e.g. housing, water and agriculture. A good example is the use of intermittent irrigation in China, India, Indonesia and Sri Lanka, where in combination with other vector control measures, it has reduced the incidence of both malaria and Japanese encephalitis, while reducing costs for farmers as this method of irrigation uses water more efficiently (*35*).

In areas where LSM is feasible, it can contribute to IVM, for example by:

- **Targeting outdoor resting and biting mosquitoes:** LSM can supplement LLINs and IRS (which target indoor adult vector populations only) by controlling vectors that are less affected by LLINs and IRS such as the vectors that rest and bite outdoors.
- **Targeting malaria 'hotspots':** In some locations malaria may persist even with high LLIN and/or IRS coverage. LSM may therefore be particularly useful in helping to remove residual foci of malaria transmission in elimination programmes.
- **Managing insecticide resistance:** Only four classes of insecticides are recommended for IRS and of these, only one class is recommended for treating mosquito nets. Anopheline resistance to all these classes has been reported (*36*). However, the wide diversity in the classes and modes of action of different larvicides, used in combination with habitat modification and manipulation, presents an opportunity to (i) reduce overall dependence on insecticides, (ii) preserve the efficacy of existing insecticides, and (iii) manage the spread of insecticide resistance once it has emerged.
- **Controlling other vector-borne diseases:** LSM can be adapted to target the vectors of other diseases, thereby improving cost-effectiveness. For example, the Indian Urban Malaria Scheme conducts LSM year-round in order to reduce the populations of vectors of dengue, filariasis and Japanese encephalitis in addition to the vectors of malaria (*37*).

1.4 When and where is LSM appropriate?

Programme managers will need to assess whether LSM is appropriate, and if so, what interventions should be used. LSM is an approach that needs to be tailored to local environmental conditions. As an example, conventional (manual) ground treatment of larval habitats is not recommended where there are extensive areas of larval habitats stretching many square kilometres, such as in areas of large-scale rice irrigation or extensive flood plains.

LSM programme design must be appropriate to the local infrastructure. In general, there are four approaches to implementing LSM:

1. LSM can be built into the national malaria control programme, in which case any country considering LSM should start on a small scale with pilot schemes and then build capacity and experience. In this context, LSM requires more than the current funding and political support: strategic, long term funding is needed so that local programmes and supporting institutions have time to learn and consolidate.
2. Small communities or municipalities with few resources but significant motivation to control malaria, such as in places where LLINs and/or IRS have not yet been deployed, can conduct LSM as part of a local community effort.
3. Sectors outside the health sector can contribute to LSM through careful road and building construction and infrastructure development, as in Khartoum (**Annex 9**).
4. Large urban areas and private schemes such as mining and agricultural operations, with an interest in malaria control and improved quality of life through reduction in nuisance mosquito populations, can implement LSM independent of, but in collaboration with, national malarial control activities using local or corporate resources.

The following factors will contribute to the success of LSM programmes (**Chapter 2**):

- **Leadership and clarity of objectives:** Personnel at all levels of the organization must receive the message that LSM is an important undertaking and has the support of the management.
- **Good management:** Management capacity is the key to a successful LSM programme. Of particular importance is the ability to quickly collate and report meaningful monitoring data. Typically it is the inadequate management of staff and logistics that limits the success of LSM programmes in suitable areas.
- **Entomologists with detailed knowledge of local vectors:** It is essential to have trained entomologists who conduct detailed surveys of the ecology and behaviour of local vectors, in order to establish which (if any) LSM interventions are appropriate and to monitor the impact of the programme.
- **Community support:** LSM interventions must have the support of the local community in a target area so that larval habitats can be accessed and either treated with a larvicide or modified. Local needs must be taken into consideration when interventions are planned, e.g. the livelihood of the local population might depend on some of the aquatic habitats (rice fields, irrigation channels and pits, wells). Therefore, educational programmes need to be implemented prior to interventions and ideally community members will be directly involved in conducting LSM, as in Khartoum (*38*), Mauritius (*39*) and Dar es Salaam (*40*).
- **Collaboration between sectors:** LSM often overlaps with the responsibilities of other sectors and therefore careful coordination can be productive and reduce costs. For example, since 2002 the Malaria Free Initiative in Khartoum State, Sudan, has coordinated with the Public Works Department (to repair broken water pipes which are an important source of vector larval habitats), the Farmers' Union and the Ministry of Agriculture (to promote intermittent irrigation), the Ministry of Education (to involve schoolchildren directly in LSM) and the media (to increase radio and television broadcasts to raise public awareness and support for the campaign). Collaboration with other sectors to ensure good practice in infrastructure development and

housing is also important, so that activities such as road construction, brick making or house building do not create new larval habitats.

- **Targeting LSM in urban areas:** Larval habitats in towns and cities are largely man-made and relatively easy to identify and treat, as in Khartoum and Dar es Salaam. In addition, other interventions, which may be acceptable to a rural population, may not be well received in urban areas, such as IRS in India, where LSM is the mainstay of urban malaria vector control. In Zanzibar, where IRS was deemed not feasible in the urban area of Stonetown, due to the density and structure of houses coupled with very low levels of malaria transmission, the malaria programme decided to plan for larviciding in this area.
- **Conducting general mosquito abatement rather than anopheline control alone:** A general reduction in nuisance biting by mosquitoes will generate support for the programme from the local population and can simplify LSM as differentiation of vector species by field staff is less necessary. Infrastructure for the control of culicine mosquitoes is important where arboviruses are a potential public health problem.
- **Strong surveillance systems:** Continuous entomological monitoring is crucial to ensure that all larval habitats are being correctly handled, and epidemiological surveillance is important to monitor the impact of the LSM programme.

CHAPTER 1
Selecting LSM interventions

This chapter describes the main types of LSM, how to assess whether LSM is appropriate in a specific location, and how to decide which LSM interventions to use.

It is recommended that environmental management (habitat modification and manipulation) should, where feasible, be the primary strategy to reduce the availability of larval habitats. Sites that should be targeted for larviciding are (i) habitats that cannot be removed through environmental management and (ii) habitats identified as candidates for environmental management but not yet modified. These will be larval habitats that are relatively few, fixed and findable.

1. Types of LSM

LSM is the management of aquatic habitats (water bodies) that are potential larval habitats for mosquitoes, in order to prevent the completion of immature stages of mosquito development, the egg, larvae and pupae (**Annex 1**). There are four categories of LSM (5), which are described in greater detail in **Chapter 3**:

- **Habitat modification**

  A permanent alteration to the environment, aimed at eliminating larval habitats, including:

  - Landscaping, surface water drainage, filling and land reclamation.

- **Habitat manipulation**

  Temporary environmental changes to disrupt vector breeding, including:

  - Water-level manipulation, e.g. flushing, drain clearance to eliminate pooling;
  - Shading or exposing habitats to the sun depending on the ecology of the vector (see **Annex 1**, **Table 1**).

- **Biological control**

  The introduction of natural enemies into larval habitats, including:

  - Predatory fish
  - Predatory invertebrates
  - Parasites or other disease-causing organisms.

- **Larviciding**

  The regular application of biological or chemical insecticides to water bodies, including:

  - **Surface oils and films**, e.g. highly refined oils and biodegradable ethoxylated alcohol surfactants, or "monomolecular films" (MMF) that suffocate larvae and pupae;
  - **Synthetic organic chemicals**, e.g. organophosphates that interfere with the nervous system of immature larval stages, such as chlorpyrifos, fenthion, pirimiphos-methyl and temephos;

- **Bacteria**, e.g. *Bacillus thuringiensis* subsp. *israelensis* (Bti), and *Bacillus sphaericus* (Bs) that produce insecticidal crystal proteins which, when ingested by larvae, attack the gut lining causing cessation of feeding and subsequent mortality;
- **Spinosyns**, e.g. metabolites extracted from the bacterium *Saccharopolyspora spinosa*, that act as nicotinic acetylcholine receptor (nAchR) allosteric activators and can cause mortality through both contact and ingestion;
- **Insect growth regulators**, e.g. diflubenzuron, methoprene, novaluron and pyriproxyfen that prevent emergence of adults from the pupal stage.

2. Analysis for LSM in a specific setting

Integrating LSM into a malaria control programme is a step-wise, information-based process. These steps should be followed systematically in order to reach a sound decision about (i) the feasibility of LSM in the local setting and (ii) which LSM interventions are appropriate.

The scale of LSM will vary in different settings, with extensive LSM in some settings and minimal LSM in others. For instances, in urban and semi-arid areas, the potential for achieving high levels of vector suppression with LSM has been demonstrated (*24,41,42,43*). In other settings, LSM may be less appropriate or totally inappropriate. In all cases, good decisions cannot be made without a systematic approach and a thorough understanding of the local vector and transmission ecology.

Information on the following factors is required to make an informed decision about whether LSM is appropriate:

- **Malaria epidemiology** (e.g. incidence of parasitologically confirmed malaria cases and determination of place of infection e.g. infections detected by urban health centres may have been acquired in rural areas).
- **Existing malaria interventions** (e.g. LLIN coverage, IRS coverage, access to diagnostic testing and treatment).
- **Vector bionomics**
  - Primary and secondary vector species (e.g. *An. gambiae* s.s., *An. arabiensis*, *An. funestus*);
  - Adult mosquito biting and resting habits (e.g. endophilic or exophilic, and endophagic or exophagic).
- **Larval ecology** (e.g. nature and stability of larval habitats) and physical environment (e.g. rural, urban, semi-arid).
- **Status of vector resistance** (e.g. pyrethroid, organophosphate, DDT or carbamate resistance; metabolic or target site resistance).
- **Economics** (e.g. predicted cost per person protected by LSM per year, potential sources of funding for LSM).
- **Health system and national malaria control programme** capacity and commitment.

Throughout the duration of baseline data collection, it is important to remember that the *primary LSM intervention should be source reduction of larval habitats* through habitat manipulation or modification. Where larval sources cannot be reduced by applying these strategies, and in sites identified for, but not yet under, environmental management, *larviciding* or *biological control* can be used to reduce mosquito production. Opportunities for environmental management (habitat management or manipulation) should always be sought for the long term.

2.1 Current malaria epidemiology, vector ecology and interventions

Before introducing LSM, it is important to make sure that the first-line interventions (LLINs and/or IRS) have achieved high coverage in the target area. Routine health system and malaria indicator survey (MIS) data on LLIN and IRS coverage or data on the number of LLINs distributed or houses sprayed, if available, may be used as a baseline. Vector susceptibility to LLIN and IRS insecticides, as well as resting and biting behaviour, should also be factored into the assessment of maximum benefit. Community acceptance of first-line interventions should also be considered. Only after the first-line intervention has achieved its maximum potential benefit, or is at risk due to biological or social factors, should LSM be considered.

To identify the areas where LSM may add benefit, the population at risk of malaria should be mapped. If possible, the proximity of households to larval habitats should also be determined. This is important since it has been repeatedly shown that people living close to larval habitats are at a greater risk of malaria (*44*).

Health management information systems (HMIS) data can be used to identify the highest burden districts, using metrics such as incidence of confirmed malaria cases (inpatient or outpatient) and test positivity rate (slide or RDT positivity rate). In areas of moderate to high transmission, the total number of malaria cases, inpatients and deaths recorded at health facilities per month can be used to assess the geographical distribution of the disease (*45*). In low endemic areas, geographical clustering of cases, or foci of transmission, should be defined and mapped because these are ideal targets for LSM.

Routine health facility data are less expensive to obtain than survey data and also help to build capacity. However, in some situations cross-sectional surveys to determine parasite prevalence may be required where health facility data are not sufficiently complete. Care must be taken to select a representative and sufficiently large sample of the target population. (For guidance on malaria surveillance and surveys, see *46,47,48*.)

Surveys and analysis of health information systems data should aim to address the following questions:

- **What are the parasite prevalence and incidence of confirmed malaria?**
- **Are there clear hotspots of malaria transmission?**
- **Which households or areas have the greatest risk of malaria infection, and is the risk greater close to certain larval habitats?**

This information should be used to identify areas or municipalities **where LSM should be first considered**, which may be characterized by:

- The highest relative malaria burden, historically or currently, in a normally low to moderate transmission area;
- A re-emerging malaria problem;
- A high population density (e.g. urban and peri-urban areas) or a low population density (e.g. rural villages) with clearly defined, limited and accessible breeding sites;
- Other vector-borne diseases.

2.2 Vector bionomics

Once potential target areas for LSM have been identified using data on malaria transmission and intervention coverage, the local vector biology needs to be well understood in order to decide which, if any, LSM interventions are suitable, and when these should be implemented. More details on the types of larval habitat suited to LSM is provided later in this section.

Search of historical data

At the outset, it is important to clearly define the target vector populations and understand as much as possible of their ecology. The first step in this process is to conduct a search of historical data regarding both adult and larval populations within the specific area targeted for protection or within similar ecotypes. This information should then be combined with data from on-going surveys of both adult and larval populations. Much of this information already exists for many operational settings, which gives a good starting point for local data collection.

Adult mosquito surveys

The purpose of adult population surveys for LSM is to confirm the presence of vector species and where these populations are concentrated. This will help focus the larval survey on more likely targets. These surveys also provide a baseline against which to gauge success of LSM interventions. It is important to remember that the measure of success for a larval control operation is the impact on the *adult* vector population; larval surveys alone are not sufficient. (For details on conducting adult mosquito surveys see **Annex 2**.)

With the aid of historical information about vector populations and current information from on-going adult surveys, it is possible to design and carry out a programme of larval surveillance and habitat identification. The information from larval surveys is of general use for other areas of a malaria control programme, not only for LSM. For example, it can help delineate malaria risk areas, forecast the need for adult mosquito control, assess the effectiveness of adult control measures, help interpret adult mosquito surveillance data, and serve as a source of specimens for insecticide resistance monitoring (*49*). (For details on conducting larval mosquito surveys see **Annex 3**.)

2.3 Physical environment

Human activity or development projects often create larval habitats. The target area should therefore be checked for specific features favouring malaria vectors, such as irrigation systems, man-made containers (for some vector species), quarries, construction sites, or borrow pits that may be used for brick making or plastering, seepage from dams, poor waste water management or broken water pipes, and urban agriculture. Other forms of larval habitats might be small ground pools resulting from ground depressions filled with rain, or low water table.

Ideally, the most productive larval habitats (those which produce the most adult Anopheles mosquitoes) should be identified so that they can be targeted for LSM. This can be done by determining the characteristics of habitats without vector larvae and those with larvae, particularly in the late instar and pupa stages. Studies have been conducted in different ecosystems to assess mosquito productivity in different water habitats (*10,12,23,29,30,50–53*). Such findings can be used to assess the feasibility of LSM and to target the intervention.

Irrigation systems

Irrigation can create larval habitats for malaria mosquito vectors, as observed in Burundi and Kenya, where irrigation greatly increased vector numbers and malaria transmission (*54*). However in other settings, transmission levels in communities close to irrigated areas are similar to, or less than, those in surrounding communities with no irrigation (*54*). The mechanisms driving this phenomenon are incompletely understood, but it is hypothesised that (i) rice production may increase disposable income, which is protective against malaria by facilitating the purchase of LLINs and antimalarial medicines, and that (ii) improvements in housing and nutrition may be significant as both can reduce malaria incidence (*54*). In some areas irrigation may change the vector community, e.g. *An. funestus* has been displaced in some locations by *An. arabiensis*, a less efficient vector.

If irrigation increases vector breeding, then simple changes such as flushing canals or intermittent irrigation may be suitable interventions. The following questions should be addressed:

- *Has irrigation raised the height of the water table and/or created standing water around the irrigation system?*
- *Has irrigation altered soil or water salinity?*
- *Is vegetation growing in channels responsible for pooling and standing water? Can removal of vegetation lead to fast-flowing water? Depending on the vector species – does vegetation increase or decrease the risk of vector breeding?*
- *What is the effect of irrigation on (i) relative species abundance and (ii) overall density of larval and adult vectors*
- *Could changes to irrigation practices be introduced, such as vegetation clearance, flushing or regular drying of canals?*

EXAMPLE: in Khartoum, Sudan, regular drying of irrigated fields, which reduces vector breeding in this setting, is compulsory in both government and private irrigation schemes. This initiative is supported by the Farmers Union and the Ministry of Agriculture, and 98.2% of irrigation schemes were dried for at least 24 hours during 2011 (*54*). Leakages from irrigation canals are also repaired and vegetation around canals is cleared in cooperation with the Ministries of Irrigation and Agriculture (*55*).

Dams

Over the past half century, an estimated 40,000 large dams and 800,000 small dams have been built worldwide (*56,57*). Such constructions can create larval habitats for malaria vectors, often with a consequent rise in malaria incidence if there are human settlements close by. The following questions should be addressed:

- **Has the dam created vector larval habitats?**
  In Tigray, Ethiopia, a microdam was associated with the creation of larval habitats, through seepage at the dam base (28% larval habitats), leaking irrigation canals (16%), pools that formed along the bed of streams from the dam (13%), and man-made pools (12%). Consequently, in the village close to the dam, there were 5.9–7.2 times more adult vectors (*An. arabiensis*) compared with a control village 3–4 km distant. There was also a 3.1% higher prevalence of splenomegaly in children under 10 years of age in the dam village (*58*).
- **Could environmental management be easily conducted?**
  In Deba village, Tigray, Ethiopia, the community was involved in filling, draining and shading of potential larval habitats and this produced a 49% (95% CI=46.6–50.0) relative reduction in *An. arabiensis* adults (*58*). In Kenya, abandoned fish ponds often become highly productive larval habitats and these could be readily filled in (*59*). Health considerations need to be considered in any development projects that involve water.

Urban infrastructure: city planning, drainage, roads and construction projects

Road construction can create larval habitats, both in the borrow pits used to obtain soil during construction and also in the drainage channels alongside completed roads, where debris often collects. Vectors also breed in concrete water storage containers used to provide water for construction projects. Poor waste water management and drainage in urban areas can create larval habitats, e.g. in Dar es Salaam, over 70% of larval habitats are man-made, of which many are drains (*22*). Therefore, there is a need for sectors outside health care, such as municipal public works departments, to take responsibility for careful construction projects and good design and maintenance of roads and drains. The following questions should be addressed:

- **Do broken water pipes, drains or cisterns provide larval habitats?**

- **Can these be easily repaired, removed, covered or treated?**
  In Khartoum, broken water pipes constitute a major larval habitat. Therefore the Malaria Free Initiative (MFI) collaborates with the Public Works Department (PWD) to repair broken water pipes. MFI is responsible for surveillance, reporting and transportation and the PWD provides engineers and equipment. By 2004, 3818 metres of water pipes had been replaced and 6104 metres repaired (55). Similarly, following drain clearance in Dar es Salaam, the probabilty of malaria infection in the local population was reduced in comparison to the pre-intervention period (OR = 0.12, 95% CI 0.05–0.3, $p < 0.001$) (*40*).

- **Are vectors breeding in other man-made structures?**
  In Malindi, Kenya, more than 90% of all larval habitats are man-made, of which many are unused swimming pools, drains, and wells. The unused swimming pools are normally located in up-market residential and tourist areas and quickly fill with water during the rains, allowing vectors to breed (*60*). These swimming pools could be filled in or treated with larvicide.

Urban agriculture

Growing vegetables and crops in urban areas may create mosquito breeding habitats, as demonstrated in Accra (*61*) and Dar es Salaam (*62*), with a consequent increase in malaria, as seen in Ghana (*63*) and Côte d'Ivoire (*64*). The following questions should be addressed:

- **Is urban agriculture creating larval habitats?**
  The methods used for cultivating crops, including the water applied, will influence the availability of larval habitats. For example, small water impoundments constructed for irrigation or soil furrows may become larval habitats.

- **Can farming practices be altered to incorporate LSM?**
  In Kampala, flooding of sweet yams was practised and provided ideal larval habitats for local vectors, although the yield of sweet yams is lower in saturated soils. In this case, discussion with farmers about best agricultural practises would be helpful to improve yield and reduce malaria transmission.

- **What are the alternatives to urban agriculture?**
  Urban agriculture provides food and commerce for local citizens and it may be difficult to introduce changes.

- **Can LSM be included in agricultural Integrated Pest Management?**
  Farmers may already be trained to identify and control pests such as diamondback moth larvae, which are pests of cruciferous vegetable crops. Farmers may also be trained to recognize and eliminate anopheline larvae in dug wells or rain-filled furrows, an approach recently explored in Kumasi, Ghana (*65*).

2.4 Status of vector resistance

LSM may be a useful component of insecticide resistance management in settings where vectors develop resistance to insecticides used for LLINs and IRS. Organophosphates are the only class of insecticide that can be used for both adulticiding with IRS and for larviciding. While organophosphate resistance in Africa is relatively rare at this time, there are other classes of bacterial larvicides – benzoylureas, juvenile hormone mimics and spinosyns – that can be used as larvicides. The insecticide susceptibility status of the local vector populations should be monitored as a part of every vector control operation. The protocol for testing larvae is available from WHO (*4*).

2.5 Economics

The costs of LSM must be carefully considered. The cost per person protected by larviciding per year is determined as follows:

- The type of larvicide formulation or equipment used

- The ratio of human population density to larval habitat density
- The potential for targeting larvicides in space and time.

Example: the cost of larviciding programmes was estimated in three different ecological settings at between US$ 0.94 and US$ 2.50 per person protected (*66*), a cost that is comparable to that of LLINs and IRS in these settings. However, cost effectiveness data for larviciding programmes across different ecological and transmission settings is lacking, making general comparisons with LLINs and IRS difficult.

Estimating the cost of a planned LSM programme

Guidelines on estimating the costs of a vector control programme are available (*67*).

The cost per person in target area per year for a planned LSM programme can be estimated by the 'ingredients approach':

- Identify the different activities to be costed;
- Quantify the 'financial' and 'economic' cost of conducting these activities. The economic cost differs from the financial cost in that it takes into account all resources used, including volunteers and donations in addition to the 'opportunity' costs of existing resources (such as equipment or buildings already owned or staff already employed);
- Summarize economic and financial costs in a table;
- Estimate size of target population for protection;
- Calculate costs per person in target area per year.

Example: a fictitious LSM programme was designed in Vihiga District in the western Kenya highlands, to protect a population of 609,324 (**Table 1**) (*66*). Costs were estimated based on a programme targeting larval habitats with larviciding for four months during the rainy season (**Table 2**). Cost per person in target area per year was then calculated (**Table 3**).

TABLE 1. **Target location summary for fictional LSM programme in Vihiga District, Kenya (*66*)**

TARGET LOCATION		DEFINED TARGET AREA FOR COSTING LSM PROGRAMME			
CITY OR DISTRICT	DESCRIPTION	ADMINISTRATIVE AREA COVERED	TOTAL POPULATION	AREA IN KM^2	POPULATION DENSITY/KM^2
Vihiga District	Rural highlands	Vihiga District (total 6 divisions)	609 324	563	1 082

2.6 Health system and national control programme capacity

LSM, like LLIN distributions and IRS programmes, is a major financial and technical undertaking, for which full political and community support is essential. Therefore it is important to assess whether the national health system and the national malaria control programme have the capacity to support LSM, and whether different sectors can work together (particularly health, agriculture, education, food and water). Moreover, programmes should assess whether they have the necessary personnel and time to implement LSM. Integral to the success of LSM will be a critical mass of trained field staff and public health officers supported by entomologists with detailed knowledge of local transmission ecology and vector control.

Even more important than entomological capacity is management capacity, particularly the ability to collate, synthesize and report monitoring data in days rather than weeks. Ensuring the entomological expertise required is often not insurmountable but large-scale programme management of logistics and human resources is usually the most limiting capacity.

In particular, the following questions should be asked:

- *What funding is available for LSM?*
- *Will funding LSM divert resources away from primary methods of vector control (LLINs and IRS)?*

TABLE 2. **Predicted staff structure for a fictional LSM programme per person per year in Vihiga District, western Kenya highlands (*66*)**

ADMINISTRATIVE LEVEL	NUMBER OF STAFF		TIME CONTRIBUTED/ PERIOD EMPLOYED PER YEAR	ROLE AND RESPONSIBILITY
Part time contribution to programme				
National or international	LSM expert	1	5 months	Advice on technical and programme management.
National	Director NMCP	1	1 week	Approval of programme, reading progress reports, signing documents, site visits
	Entomologists	1	1 week	Reading progress reports, technical advice, site visits
	Procurement Officer	1	1 week	Central procurement of larvicide and equipment
Provincial	Malaria Control Officer	1	1 week	Engagement in stakeholder meetings. Site visits with NMCP staff
District	Public Health Officer	1	1 hour/week	Involvement in weekly management meetings
	Medical Officer	1	2.5 weeks	Involvement in weekly management meetings, sensitization of district health staff
Full time programme staff				
District	Programme manager	1	12 months	Day to day programme management, financial management. Recruiting and training divisional heads
	Driver	1	12 months	Transport of programme manager, transport of equipment and supplies
	Administration assistant	1	6 months	Recruitment and supervision of larval control personnel (LCP), larval habitat spot checks
Division	Divisional heads	6	12 months	Field work management, quality control, adult mosquito monitoring, reporting to programme manager. Evaluation and planning subsequent year's intervention. Training of supervisors and LCP.
Location	Supervisors	26	6 months	Recruitment and supervision of LCP, larval habitat spot checks
Valleys	LCP	367	18 weeks	Treating larval habitats with larvicide, reporting to supervisors, collecting larvicide from divisional store

- *How is the health system organized at district, regional and national level and does it have the capacity to support or implement LSM?*
- *Are health system and control programme managers in favour of LSM?*
- *Are there sufficient staff, in particular trained entomologists, technicians and field staff?*
- *Is there capable management for LSM?*
- *Is there sufficient logistical support?*
- *Are there outside sources that may provide initial entomological, management and logistical support while building capacity at the local level?*
- *Could existing monitoring and evaluation activities be adapted or streamlined to work for LSM?*

TABLE 3. **Predicted financial and economic costs of a fictional LSM programme per person per year in Vihiga District, western Kenya highlands**

Predicted costs using VectoBac CG formulation (in US$ 2006 at midpoint larvicide price) (*66*)

COST CATEGORY	PRE-IMPLEMENTATION COSTS (Y0) TOTAL:		IMPLEMENTATION YEAR COSTS (Y1) TOTAL:		AVERAGE ANNUAL COSTS:		PROPORTION OF TOTAL AVERAGE:	
	FINANCIAL COST	ECONOMIC COST	FINANCIAL COST	ECONOMIC COST	FINANCIAL COST	ECONOMIC COST	FINANCIAL COST	ECONOMIC COST
Recurrent costs								
International staff costs	23 013	23 013	34 665	34 665	37 541.7	37 672.5	0.04	0.04
NMCP/MoH staff costs	0	0	0	60 001.1	0	60 001.1	0	0.07
Programme staff salaries	11 484.6	9 848	110 894	95 091.6	112 329.5	96 378.6	0.12	0.11
Larvicide (CIF) protective cloth	0	0	625 985.3	616 959.8	625 985.3	616 959.8	0.69	0.67
Staff training	0	0	12 820.4	10 769.2	12 820.4	10 769.2	0.01	0.01
Community sensitization	0	0	68.9	57.8	68.9	57.8	0	0
Operations costs and overheads	1 985.5	1 667.8	81 493.1	68 454.2	81 741.3	68 672.1	0.09	0.07
Transport	6 324.8	5 312.9	14 081.8	11 828.7	14 872.4	12 523.1	0.02	0.01
Adult mosquito monitoring	799.8	671.8	1 599.6	1 343.7	1 699.6	1 431.5	0	0
Capital costs								
Maps	66.7	83.8	66.8	83.8	75	94.8	0	0
Vehicles	5 095.1	4 636.2	10 190.3	9 272.5	10 827.2	9 878.4	0.01	0.01
Spray pumps	0	0	0	0	0	0	0	0
Computers, other equipment	871.7	774.9	2330	2072.4	2439	2 173.6	0	0
Adult monitoring equipment	200	183.4	400	366.8	425	390.8	0	0
Subtotal recurrent costs	43 607.7	40 513.6	881 608.1	899 171.1	887 059.1	904 465.7	0.98	0.99
Subtotal capital costs	6 166.8	5 594.5	12920.3	11711.6	13691.1	12 442.8	0.02	0.01
Total costs of programme	49 774.5	46 108.1	894 528.4	910 882.7	900 750.2	916 908.5	1	1
Cost per person protected	0.08	0.08	1.47	1.49	1.48	1.5		

3. Information analysis

When as much baseline information has been collected as possible, it should be summarized as follows:

Malaria epidemiology	What is malaria incidence and parasite prevalence?
	Are there transmission foci?
Demography	How high is population density and which are the high density areas?
Other interventions	What other interventions are on-going in the area?
	If IRS and/or LLIN coverage is not high, why is this? Is it due to a lack of input from the government? Is it because people are nomadic, so do not have permanent houses?
Available resources	What staff are available or could be made available to conduct LSM?
	How much funding is available for LSM?
Larval habitats	Primary types of larval habitats that affect the protected area
	Extent of larval sources (number and size) that affect the protected area
	Are there other larval sources that are difficult or impossible to reach in the area?
	Changes in the size, number, location and density of different larval stages in these sources over the course of the year (rainy vs dry season)
Adult vector ecology	What are the primary vector species?
	What are the typical vector biting and resting habits?
	What is the status of vector resistance to insecticides used for IRS/LLINs?
Potential for LSM	Impact of human activity on occurrence or mitigation of larval sources
	Proximity of larval sources to human habitation
	Potential for modification of larval habitats to reduce or eliminate mosquito development
	Potential for treatment of sources with mosquito larvicides
	Potential for natural or introduced predators to augment control
	Practicality of finding and treating larval sources at various points in the year
	Expected area needing treatment (total hectares per year) under dry season, rainy season, and full year treatment

4. Decision-making: feasibility of LSM and possible approaches

Using the information collected above, a decision can be made about the feasibility of LSM.

Feasibility of LSM in broad eco-epidemiological settings

In general, LSM will likely be most cost effective and efficacious in locations where larval habitats are relatively few, well-defined, seasonal, readily accessible without aerial equipment, and possibly man-made (i.e. 'few, fixed and findable'); and in more temperate regions where larval development is more protracted. Such conditions are common in areas of low to moderate, focal or epidemic transmission. These conditions occur frequently in urban areas, in desert fringes, at high altitude and in rural areas with high population densities.

LSM is not a strategy for application in all habitats and is not a stand-alone intervention. However LSM could be integrated into malaria control or general mosquito abatement programmes once transmission has been reduced to low or moderate levels by LLINs or IRS, or once these interventions have reached their maximum practical effect. LSM might therefore be advocated for the pre-elimination and elimination phases of malaria control, alongside LLINs and IRS, where it may be targeted in space and time. LSM may also have potential in managing insecticide resistance and outdoor transmission.

An overview of the likely effectiveness and challenges of implementing malaria vector control, including larviciding, in seven eco-epidemiological types is given in **Table 4** (*68*).

The WHO Interim Position Statement (2012) on the role of larviciding in sub-Saharan Africa (*3*) recommends the following settings as being potentially suitable for larviciding:

Urban areas: where breeding sites are relatively few, fixed and findable in relation to houses (which are targeted for LLINs and IRS).

Arid regions: where larval habitats may be few and fixed throughout much of the year

East African Highlands: where a field trial in 2009 (*21*) demonstrated that larviciding can be effective in conjunction with LLINs.

TABLE 4. **Suitability of LSM in different eco-epidemiological zones (68)**

AREA	MAIN VECTORS	MALARIA EPIDEMIOLOGY AND CONTROL	IS LSM POTENTIALLY SUITABLE?
Steady state ecosystems			
Rural, tropical African savannah and savannah belts merging into equatorial rainforest	*An. gambiae, An. arabiensis*	Transmission is generally low but foci remain, despite LLINs and/or IRS. Population density is relatively low with well-defined and few larval habitats, or population density is high with well-defined habitats.	Yes
		Good LLIN coverage yet high insecticide resistance or outdoor biting. Parasite prevalence 50%–80% in children aged <5years, annual EIR 50–350. Population density is relatively low with well-defined and few larval habitats, or population density is high with well-defined habitats.	Yes
		Good LLIN coverage yet high insecticide resistance or outdoor biting. Transmission was historically high but has been reduced to <20% parasite prevalence in children aged <5years, annual EIR<10. Population density is relatively low with well-defined and few larval habitats, or population density is high with well-defined habitats.	Yes
		Intense, perennial or seasonal, influenced by rainfall and local ecology. Parasite prevalence 50%–80% in children aged <5years, annual EIR 50–350. Population density is relatively low with numerous and diffuse larval habitats and/or low coverage with LLINs and IRS and/or little insecticide resistance or outdoor biting.	No
Forest and forest fringes	*An. gambiae* s.s.	Large variation in ecology of malaria transmission.	Yes, in relatively permanent settlements.
Highland and desert fringes	*An. arabiensis*	Areas between highly endemic regions and deserts or high altitude areas with no transmission. Seasonal with risk of epidemics increasing as endemicity declines. Population density relatively low with well-defined and few larval habitats, or population density high with well-defined habitats.	Yes
Wetland and coastal areas	*An. melas* (West Africa); *An. merus* (East Africa)	Transmission affected by: climate, demography, environment and land use, human behaviour, development.	Yes
Urban and peri-urban areas	*An. arabiensis*	Transmission generally lower than rural areas.	Yes
Situations of rapid development change			
Agricultural development projects			Yes

The WHO Statement also highlights the importance of assessing beforehand the feasibility of conducting and sustaining LSM in these settings.

Feasibility of LSM in a specific setting

The feasibility of LSM as a tool in malaria vector control programmes should ultimately be determined locally. Expected resource requirements for LSM will need to be considered relative to the potential reduction in transmission intensity or disease incidence (if possible). It is important to consider that while complete control or elimination of all larval sources potentially affecting the control area is preferred, benefits may be realized from alternate, more focused interventions. Possible scenarios are outlined later in this section.

To determine the feasibility of LSM, the following questions should be addressed:

- *Where and when is LSM indicated?*
- *Can LSM be focused to protect populations at risk?*
- *Are there operational synergies with existing interventions?*
- *Can LSM be integrated into sectors outside healthcare?*
- *Is there an opportunity to increase knowledge at the district public health office level in order to initiate and improve LSM at the community level?*
- *Is there sufficient funding for LSM? From what level could funding be provided (national only or also at a more decentralized level, e.g. district health management teams)?*
- *Is there sufficient funding to target both anophelines and other mosquito species such as culicines (which is ideal if practicable and affordable)?*
- *Will LSM be cost effective? While not strictly a sub-category of "feasibility" the cost-effectiveness of LSM as a supplement to other vector control should be evaluated.*

Malaria control programme managers may contact WHO for further technical assistance in determining the feasibility of LSM in a specific setting.

5. Possible LSM strategies

Wherever LSM is implemented, the order of priority for different LSM interventions is as follows:

1. Source reduction of larval habitats should be conducted wherever possible, through direct action and community mobilization to achieve habitat manipulation or modification.
2. Where larval sources cannot be reduced or during periods between identification of the larval sources and implementation of source reduction, larviciding (or biological control) can be used to control mosquito development in these habitats.

5.1 Strategy 1. Community-led environmental management

Environmental management (habitat manipulation and modification) is the priority LSM strategy. Highly productive habitats, especially close to housing, should be targeted for source reduction as quickly as possible and as financially feasible. Elimination of larval sources within the immediate vicinity of housing should be practised whenever possible (*51,69,70*). This can often be achieved by educating the community about LSM and about common misconceptions regarding rubbish and vegetation clearance, i.e. anophelines do not oviposit (lay eggs) in rubbish and clearing brush or vegetation around houses does not reduce anopheline biting. Special lessons in schools might be introduced through collaboration with the Ministry of Education or by approaching schools directly. To educate the wider population, meetings with individual communities or awareness campaigns through the media should be conducted. Individuals can take responsibility for the elimination of small larval habitats near the home and this community ownership of LSM at an

early stage will help if larviciding is to be introduced later. Laws may be useful, for example in Khartoum, Sudan, water basins are removable by law as they provide larval habitats, and irrigation of agricultural land must be intermittent (**Annex 9**). However, it is often difficult to achieve source reduction objectives through community participation alone. Major larval habitats may require direct action by the relevant government agency.

5.2 Strategy 2. Large-scale environmental management

In some circumstances where larger scale engineering is required to achieve environmental management (**Annex 7**), communities will be unable to carry out LSM alone. Therefore collaboration with other ministries can be fruitful, as in Khartoum, where the Water Corporation Department is responsible for the repair of broken water pipes that leak and provide larval habitats (**Annex 9**). Careful design and maintenance of infrastructure is also important, e.g. small barriers built in roadside drains to slow the flow of water can create breeding sites.

5.3 Strategy 3. Larviciding of all potential larval habitats

Once environmental management has been used to eliminate as many larval habitats as possible, larviciding can be considered. Applying larvicides to all potential breeding sites is labour-intensive, but can be cost effective in urban centres (*24,63*). In areas with extensive larval sources such as large river floodplains and rice production areas, larviciding may be impractical. However, larviciding can be targeted at the most productive larval habitats which might have been difficult to eliminate through habitat modification. Other situations in which larviciding could be used include habitats with low numbers of vectors or productive habitats but of limited longevity during the rainy season only e.g. in areas of brief seasonal transmission. Population suppression of larval habitats through larviciding may also be possible in the dry season, especially in very cool seasons as in parts of southern Africa, or at the beginning of the rainy season.

5.4 Strategy 4. No introduction of LSM

The choice of not introducing LSM into a malaria vector control programme is often the correct choice, particularly in wet rural areas or in areas with vast larval sources such as large river floodplains and extensive rice planting areas. As with all of the scenarios discussed, this option should be periodically reviewed in light of current scientific information and changing local conditions. For instance, elimination of a highly productive larval source from within a village could have a significant impact on dry season transmission. Such spot treatments should never be completely ruled out.

5.5 Strategy 5. Anopheline and *culicine control (general mosquito control)*

Targeting all mosquito species rather than aiming specifically to control Anopheles is highly advisable wherever resources allow, because:

- Few people understand that only anophelines transmit malaria;
- LSM programmes that include *Culex* and *Aedes* mosquitoes often have good popular support as they improve quality of life through general reduction in overall mosquito biting;
- Field staff have less need to differentiate between genera when surveying and conducting LSM;
- IVM envisages the control of more than one vector-borne disease. Larviciding in urban areas may help control malaria, lymphatic filariasis, dengue and other mosquito-borne diseases as well as reducing nuisance biting;
- For example, the Dar es Salaam UMCP targets both culicine and anopheline mosquitoes to reduce nuisance biting and maintain community support (*22*).

6. Selection of interventions

Choosing which LSM intervention to implement and which materials to employ are important decisions, and must be based on the specific setting. The typical larval habitats of the major malaria vectors in Africa are described in **Annex 1**.

When choosing interventions it is also important to consider the following:

- "Efficacy" of the intervention: whether it will reduce adult vector populations and reduce malaria transmission in the target area.
- "Effectiveness" of the intervention: whether it can be sustained beyond the pilot stage into an on-going activity.
- Cost of the intervention, including product, labour (especially if frequent re-treatments are necessary), monitoring and evaluation.
- Cost-effectiveness of the intervention: how does this contribute to malaria transmission reduction in relation to other interventions, such as IRS and LLINs?
- Environmental impact (e.g. is the dambo (a shallow wetland) a necessary water source for animals and agriculture?)
- Worker and community health safety (are only WHOPES-approved larvicides being applied?)

6.1 Habitat modification and manipulation (environmental management)

Habitat modification is a permanent alteration to the environment, including landscaping, surface water drainage, filling and land reclamation, coverage of water storage containers with mosquito-proof lids or permanent slabs and coverage of the water surface with a material impenetrable to mosquitoes (e.g. expanded polystyrene beads).

Habitat manipulation is a recurrent activity including water level manipulation (e.g. stream flushing, keeping drains clear of vegetation so that water can flow too fast to support mosquitoes).

Environmental management is more than habitat modification and manipulation alone; it involves the mobilization of the local population to encourage their involvement in (and sustainability of) LSM, through education of health workers and the community.

If the major larval habitats can be dealt with through environmental management, this will have a significant impact on the intensity of transmission. As discussed in more detail below, this may involve ensuring that culverts are included in road construction, and that borrow pits or rock quarries used for brick making and gravel are filled or drained.

Despite its high short-term costs, environmental management can be economical in the long term due to the longevity of its protection, especially if costs can be shared between sectors. For example, malaria was controlled in Zambian copper mines between 1929 and 1949 using vegetation clearance from the drains, river straightening, swamp drainage, oiling, house screening, some quinine prophylaxis, and a limited number of untreated bednets. The estimated cost of environmental management was US$ 858 per death averted, which falls within the range documented for ITNs (US$ 219–295) if one assumes the duration of effectiveness for environmental management is ten years and three for ITNs (*67*).

6.2 Larviciding

Larviciding is the regular application of biological or chemical insecticides to water bodies. Classes of formulation recommended by the WHO Pesticide Evaluation Scheme (WHOPES) are listed in **Table 5**. Of these classes, only certain specific products have been approved by WHOPES as being

safe, stable, potent and efficacious. Quality control for *all* vector control products, including larvicides, treated mosquito nets, and insecticides and application equipment for IRS, is a critical element of all vector control programmes. Money can be wasted and people and the environment can be harmed by poor quality or non-approved products. For updated information on recommended products and measures for quality control (*71*), see the WHOPES website http://www.who.int/whopes.

Larviciding can be an attractive method for complementing on-going malaria control programmes. The costs of larviciding may compare favourably with those for IRS and LLINs, especially where malaria transmission is moderate and focal and where larval habitats are accessible and discrete (*65*). However an intensive surveillance and treatment system is required to maintain coverage of all potential larval habitats. Furthermore not all larvicides will be appropriate for treating drinking water. The safety of methoprene, pyriproxyfen, temephos and *Bacillus thuringiensis israelensis* for use in potable water has been assessed by the WHO Programme on Chemical Safety (*72*). These products are safe and are approved for use in drinking water.

There are five main groups of larvicides: oils and surface agents; synthetic organic chemicals; bacterial larvicides; spinosyns; and insect growth regulators. Details of these are outlined below.

1. Oils and surface films

These agents include petroleum distillates and monomolecular surface films (MMF) such as isostearyl alcohol made from renewable plant oils. They act by suffocating larvae or disrupting surface tension, inhibiting the ability of larvae to rest and breathe at the surface of the water causing them to drown and interfering with adult emergence. They are considered effective in control of *Anopheles* larvae, but may be impacted by wind or absorbed by vegetation. These agents will affect any aquatic invertebrate requiring use of the air-water interface for breathing, resting or egg-laying. Re-treatment is needed weekly (5).

The application of oil to water is one of the oldest forms of larval control. Due to their relatively high cost in comparison with some other larvicides and because they have limited persistence, their use has declined in mosquito control. MMFs were developed during the 1980s and while several isostearyl alcohol products are available, these have not been used extensively in mosquito control programmes in Africa.

Advantages (5,73):

The oil is visible on water so it is easy to see where it has been applied;

- Oil is a relatively cheap and easy method of larval control for small water bodies such as borrow-pits, pools, latrines and soak away pits;
- Mosquitoes cannot develop resistance to oil;
- At recommended doses, oils and MMFs are not toxic to most non-target organisms including mammals and fish;
- Combinations of MMFs with other larvicides such as Bti may significantly increase their efficacy.

Disadvantages (5,73)

- Expensive for large-scale treatment;
- Limited effectiveness in the presence of vegetation and floating debris;
- Relatively short-lived effect;
- Oils and MMFs coat vegetation;
- Oils and MMFs are readily dispersed by wind.

2. Synthetic organic chemicals

Organochlorine insecticides were discovered in the 1940s and this led to their widespread adoption for larval control. However resistance emerged in the 1950s (*5*) and for this reason, as well as the discovery that these chemicals persist in the soil and tissues of plants and animals, they are no longer recommended for the control of larvae (although the organochlorine DDT can be safely used for IRS following WHO guidelines).

Organophosphates are synthetic organic chemicals that can kill mosquito larvae by interfering with the enzyme acetylcholinesterase, which is required to regulate nerve transmission in all organisms (*5*). Organophosphates are considered less persistent in the environment than organochlorine insecticides and are therefore still recommended by WHO. However, staff require proper training in handling the concentrated material. The organophosphate temephos has been used extensively as a larvicide against blackfly larvae in the West Africa Onchocerciasis Control Programme, against copepods in the guinea-worm eradication programme, and against *Aedes* larvae in domestic water storage containers in dengue control programmes.

Pyrethroids are toxic to fish and may select for resistance, and therefore must not be used for control of mosquito larvae (*71*).

Advantages (5):

- Operations can be carried out quickly;
- Larvicides can be applied by hand for small-scale treatments;
- For large-scale treatments, agricultural sprayers or IRS hand-compression spray pumps may be used.

Disadvantages (5):

- Control is temporary and frequent reapplication may be required;
- Some larvicides are harmful to non-target organisms including the natural predators of larvae;
- Larvicides may be toxic to humans, therefore precautions are necessary.

3. Bacterial larvicides

Bacterial larvicides (BL) include products based on the insecticidal crystal proteins produced by *Bacillus thuringiensis* subsp. *israelensis* (Bti), and *Bacillus sphaericus* (Bs). Upon ingestion by mosquito larvae, these proteins are modified by enzymes in the larval midgut and then bind with specific receptors on the midgut epithelium, resulting in pore formation and interruption of feeding and homeostasis. This unique mode of action accounts for the specificity of bacterial larvicides and their utility in managing mosquito resistance to chemical insecticides.

When manufactured under proper controls, and applied properly, bacterial larvicides present essentially no risk to the environment, workers, public health or local economies (*22*). Frequency of re-treatment with bacterial larvicides can range from 1 to 4 weeks for *Anopheles* depending on formulation, habitat, temperature, and species. Typical re-treatment intervals with Bti are 7–10 days. For maximum efficiency, the re-treatment interval should be determined by recovery of late 4th instar larvae to established thresholds, or the first appearance of pupae. Bs formulations have been demonstrated to provide longer residual activity, with up to 4-week re-treatment intervals in some habitats.

Advantages (5):

- Operations can be carried out quickly;
- Harmless to other insects, fish, birds, mammals and humans at the recommended doses;
- Safe for use in multiple habitats including drinking water and on irrigated crops;
- Effective where mosquitoes have developed resistance to synthetic chemical larvicides;
- Extensive bacterial larvicide formulation options allow for various efficacy and residual objectives at the IVM programme level.

Disadvantages (5):

- The window of time for application is narrower, relative to that for synthetic chemicals;
- Larvae must be feeding when the bacterial larvicide is present for it to be effective (for mosquitoes, this is the 1st to the middle 4th instar; very late 4th instar larvae cease feeding as they prepare for pupation);
- In open, natural habitats, Bti breaks down quickly in the environment, so more frequent applications may be needed.

4. Spinosyns

Spinosad consists of spinosyn a and spinosyn d, which are metabolites extracted from fermentation using the bacterium *Saccharopolyspora spinosa*. Spinosad acts as a nicotinic acetylcholine receptor (nAchR) allosteric activator. It is available as an emulsifiable concentrate, dispersible tablets, granules and suspension concentrate, and has very low acute toxicity to mammals.

Advantages:

- Operations can be carried out quickly;
- Harmless to fish, birds, mammals and humans at the recommended doses;
- Relatively safe for use in multiple habitats including drinking water and on irrigated crops;
- Effective where mosquitoes have developed resistance to synthetic chemical larvicides.

Disadvantages:

- Product can be used to also control agricultural pests;
- Not as target-specific as bacterial larvicides;
- Toxic to non-target aquatic invertebrates as well as other beneficial arthropods (e.g. bees) (*74,75*).

5. Insect growth regulators

Insect growth regulators (IGRs) belong to two groups (*5*):

- Juvenile hormone mimics such as methoprene and pyriproxyfen, which prevent the development of larvae and pupae into adults;
- Chitin synthesis inhibitors such as diflubenzuron and triflumeron, which kill larvae when they moult.

These products are expected to be effective based on laboratory and semi-field data, but the efficacy of all IGR formulations has not yet been fully assessed in the African environment, although trials are currently on-going. These products affect a broader range of invertebrate species than do bacterial mosquito larvicides, and may exert a broader effect on ecosystems. Juvenile hormone mimics in general have been shown to have long residual effect, so the re-treatment interval is long relative to other larvicides. More frequent re-treatment is generally required for chitin synthesis inhibitors. In general, the effectiveness of IGR formulations may last longer if applied as granules, microcapsules or briquettes in specific habitats (e.g. natural and artificial containers). In Sri Lanka, only two annual treatments of gem pits with pyriproxyfen were required as larval habitats were fixed and sheltered (*31*).

Advantages (5):

- Operations can be carried out quickly;
- Long-lasting residual impact from 2 weeks up to 6 months in specific habitats reduces re-treatment cycles;
- Highly effective at extremely low dosages;
- Relatively safe for use in drinking water and irrigated crops that have been treated, can be safely eaten;

- Effective where mosquitoes have developed resistance to synthetic chemical larvicides;
- Very low toxicity to mammals, birds, fish and adult insects.

Disadvantages (5):

- High dosages (e.g. when accidentally overdosed for mosquito control) can be toxic to the immature aquatic stages of some non-target insects and to some crustaceans;
- The impact of the treatment with hormone mimics is very difficult to monitor for the immature stages because larvae develop normally and the impact can only be observed after evaluating adult emergence from pupae; monitoring systems therefore need to be set up for IGRs than for other larvicides that kill larvae within 48h.

WHO-recommended larvicides

WHOPES-recommended formulation classes are listed in **Table 5**. Of these, only some specific products have been tested and approved by WHOPES for safety and efficacy. For updated information on recommended products, see http://www.who.int/whopes (*71*).

WHO recommendations for the use of specific pesticides are valid only if linked to WHO specifications for their **quality control** (*76*).

The **environmental impact** of larvicides and their risk to humans should also be assessed according to WHOPES guidelines (*77*).

The **efficacy** of specific larvicides against local malaria vectors should be determined prior to use in a programme, using WHO-recommended methods for laboratory and field testing (*3*).

TABLE 5. **WHOPES-recommended compounds and formulations for control of mosquito larvae (*71*)**

INSECTICIDE COMPOUNDS AND FORMULATION(S)[a]	CLASS GROUP[b]	DOSAGE (ACTIVE INGREDIENT)	
		GENERAL (G/HA)	CONTAINER-BREEDING MG/L
Bacillus thuringiensis subsp. *israelensis*, strain AM65-52, WG (3000 ITU/mg)	BL	125–750[c]	1–5[c]
Bacillus thuringiensis subsp. *israelensis*, strain AM65-52, GR (200 ITU/mg)	BL	5 000–20 000[c]	–
Chlorpyrifos EC	OP	11–25	–
Diflubenzuron DT, GR, WP	BU	25–100	0.02–0.25
Novaluron EC	BU	10–100	0.01–0.05
Pyriproxyfen GR	JH	10–50	0.01
Fenthion EC	OP	22–112	–
Pirimiphos-methyl EC	OP	50–500	1.0
Temephos EC, GR	OP	56–112	1.0
Spinosad DT, EC, GR, SC, AC	SP	20–500	0.1–0.5

[a] DT = tablet for direct application; GR = granule; EC = emulsifiable concentrate; WG = water-dispensable granule; WP = wettable powder; SC = suspension concentrate; AC = aqueous concentrate
[b] BL = Bacterial Larvicide; BU = Benzoylureas; JH = Juvenile Hormone Mimics; OP = Organophosphates; SP = Spinosyns.
3 Formulated product.

Compounds and formulations not recommended for larviciding

The following compounds and formulations are not recommended for larviciding:

- Organochlorines such as DDT are not recommended for larviciding because they persist in the environment (*78*).
- Pyrethroids are not recommended because they harm non-target arthropods and their use may select for pyrethroid resistance (*78*).

6. Biological control

Biological control is the introduction of natural enemies into larval habitats, including predatory fish, predatory invertebrates and parasites or other disease-causing organisms. Biological control can be used for malaria control in some specific settings where larval habitats are well-defined, water conditions are suitable, or when chemical larviciding is not suitable (*79*). It is preferable to use indigenous species over exotic species as they are adapted to the local environment and there is no risk of invasion.

Biological control agents include (*5*):

- Larvivorous fish (predatory)
- *Toxorhynchites* spp mosquito larvae (predatory)
- Dragonfly larvae (predatory)
- Cyclopoid copepods, (predatory)
- Nematode worms (parasitic)
- Fungi (grow in the bodies of mosquito larvae and kill them)
- Neem, an oil extract of seeds of the neem tree, *Azadirachta indica* (larvicidal)
- *Azolla*, a free-floating fern that can completely cover water surfaces (prevents breeding).

Biological control can be effective especially when combined with other interventions, such as habitat manipulation and bacterial larvicides that do not harm the biological control agents. However the use of biological control agents requires a thorough understanding of the local vector ecology. Larvivorous fish use can be cost effective, as demonstrated in California and Afghanistan, especially where the local community is involved in maintaining fish stocks (*79*). But if fish are introduced into larval habitats, impact should be assessed: there should be no intervention without evaluation.

Advantages (5):

- If introduced into a suitable environment, natural larval predatory fish may establish themselves, thereby providing a self-perpetuating method of larval control;
- Fish are not expensive to introduce, nor is specialist equipment required;
- Fish do not contaminate the environment and can be used in reservoirs of drinking water.

Disadvantages (5):

- Natural predators are only effective if a large number become established, and may never provide comprehensive control (i.e. mosquito breeding may continue at low levels), and therefore larvicides may also be necessary;
- Natural predators may take several months to control larvae;
- Fish are less effective where there is abundant vegetation or debris and these require removal;
- Fish must be reared in special ponds;
- Transportation and stocking require particular care;
- Introduction of non-native species can disrupt ecosystems;
- Apart from larvivorous fish, the other methods of biological control have not been used on a large scale to evaluate their effectiveness.

CHAPTER 2

Planning and managing LSM programmes

1. Structure and organization of LSM programmes

LSM programme design must be appropriate to the local environment and infrastructure. In general, there are four ways of implementing LSM:

(i) Small communities, private entities, or municipalities with significant willpower to control malaria, can conduct LSM as part of a local community or corporate effort.

(ii) LSM can be built into the national malaria control programme, in which case any country considering LSM should start at a small-scale with pilot schemes and then build and institutionalize capacity and experience. In this context, LSM needs more than just current funding and political support but rather strategic, long term funding so that local programmes and supporting institutions have time to learn and consolidate.

(iii) As with other key malaria interventions, collaborations between ministries can be encouraged so that LSM can be integrated into infrastructure development projects and agricultural practices. There are likely to be numerous situations where this is feasible and this approach therefore has the potential to be highly effective.

(iv) Large urban areas and private schemes such as mining and agricultural operations, with an interest in malaria control and improved quality of life, can implement LSM independent of national malarial control activities, using local or corporate resources.

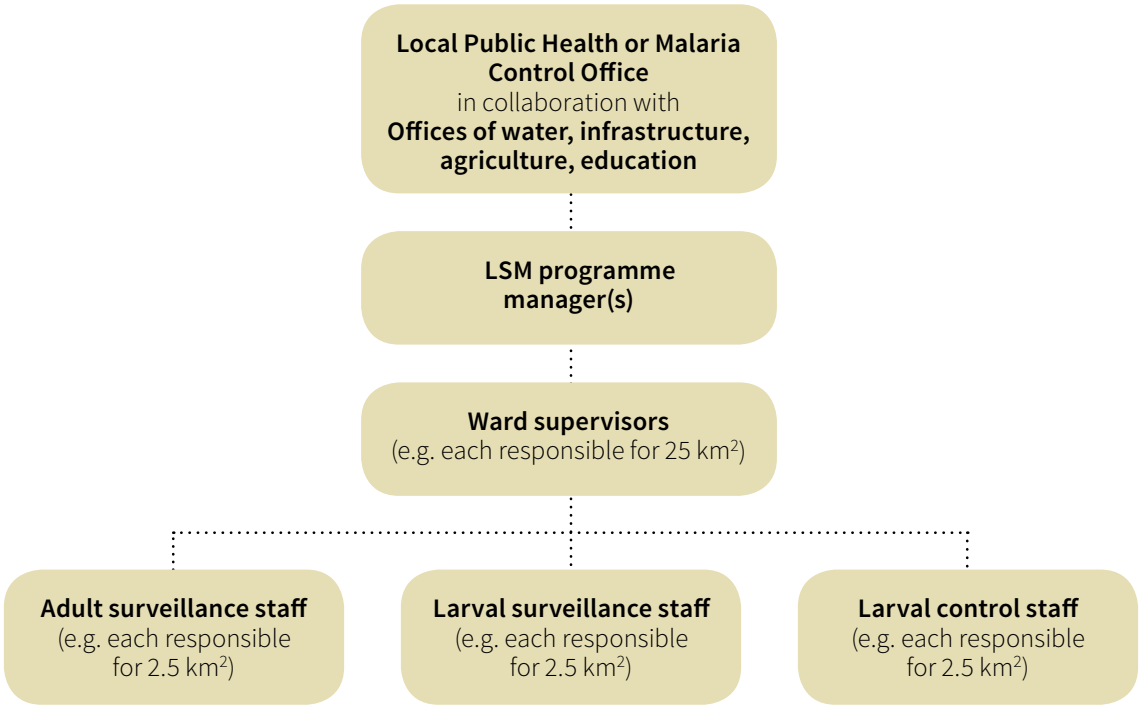
1.1 Funding

Ideally, a LSM programme will be funded by the government as part of general health expenditure, municipal public works and/or infrastructure development. Costs for the ministry of health can be kept lower by involving other ministries or corporations with economic interests in healthy workers. Costs can also be reduced by recruiting volunteers or modestly paid members of the community. School children, scouts and other service organizations can also help in LSM efforts on a few designated days each year.

1.2 Programme structure

To ensure that LSM is thoroughly and correctly conducted, a chain of command will need to be established. Specifics of the LSM programme should fit within existing programmes and take advantage of current infrastructure. Ideally a LSM programme should be operated by the local authority in collaboration with other sectors. A possible structure for a LSM programme is shown in **Figure 1**. An important point to note is that larval control and surveillance must each be conducted and reported separately, so that competing interests are minimized and quality assurance is not compromised.

Figure 1. Simplified LSM programme structure
LSM programmes are in reality far more complex and will need to be fitted into existing malaria control structures. A well thought out management structure incorporating monitoring, evaluation and management is crucial.



It is important to decide on specific roles and responsibilities (who will do what, and at what spatial scale) and how the multiple institutions directly or indirectly involved in LSM will interact. This is a key step that many LSM programmes do not achieve, with the result that it is easy for staff to take responsibility for success and to readily offload responsibility for failure. Lack of clarity of roles and responsibilities inevitably results in (i) competition between the technical and oversight staff, (ii) politicization of the technical staff at the expense of doing their day-to-day technical work, and (iii) a disconnect with staff in other sectors, especially local government.

In some situations it may be necessary to recruit new staff for the LSM programme as existing public health staff may be overburdened and unable to take on extra responsibilities. However, wherever possible LSM should be fully integrated into the existing public health infrastructure with the involvement of existing public health staff. If a malaria control programme is well established, with other vector control activities being operated by a team of field workers, it may be possible for LSM to be conducted by this team, if the team is locally-based and has local knowledge. This requires training of these field workers in LSM and some new workers may be required. If the malaria control programme does not have a team of field workers, it may be appropriate to create a new team, depending on the resources available.

Adequate staff for supervision and evaluation will be required if the programme involves extensive larviciding. An engineer may be needed if large scale environmental management operations are planned and if coordination with other sectors is envisaged. LSM should be integrated into other components of the malaria control programme as much as possible, e.g. when IRS teams are operating in a particular area, larval surveys or application of larvicide can be conducted by individuals trained in LSM if the timing is appropriate (IRS is best completed prior to seasonal rains, so teams may not always be present at the appropriate time for LSM). Careful timing of different components of the malaria control programme can help ensure staff can be employed fully at all times, for example ensuring that LLIN distributions do not coincide with intensive periods of larviciding.

Examples of LSM programmes currently in operation:

- *Urban Malaria Control Programme, Dar es Salaam, Tanzania* (**Annex 8**)
  The UMCP was initiated by Ilala Municipality, before evolving into the Urban Malaria Control Programme, supported by academic institutions and the government (*22,41*). The UMCP is fully integrated into the Dar es Salaam City Council administrative system and operates at five administrative levels: the City Council, municipalities, wards, neighbourhoods and over 3000 Ten Cell Units (TCUs) (*80*). Community-Owned Resource Persons (CORPS), modestly paid members of the community, are responsible for routine mosquito control and surveillance and report to Ward Offices, which in turn report to a chain of command ultimately led by the Ministry of Health (*22*). The malaria control programme in Dar es Salaam is a good example of a community-based LSM programme that has evolved from a small-scale, locally operated project.

- *Malaria Free Initiative (MFI), Khartoum, Sudan* (**Annex 9**)
  The Khartoum MFI is run by the Ministry of Health in collaboration with other sectors. Khartoum State is divided into working areas. The MFI employs 14 trained medical entomologists, 60 public health officers, 180 sanitary overseers, 360 assistant sanitary overseers and 1170 spray men (*55*). Each public health officer is assigned a working area, supported by the sanitary overseers and assistant workers ('mosquito men') and supplied with the necessary equipment (*38*).

- *Urban Malaria Scheme, India* (**Annex 11**)
  The UMS includes 131 towns, in each of which the UMS is run by a biologist and supervised by State and Central Health Authorities. Every target municipal area of each town is divided into wards of 25.6 km^2, which are further divided into sectors of 2.56 km^2. Each ward has one inspector and one insect collector, and each sector has one supervisor field worker and one or two field workers (depending on the quality of the drainage system). One driver and vehicle is provided per 40 sectors (*22*).

- *Urban Malaria Control Programme (UMCP), Malindi, Kenya*
  The UMCP was initiated in 2008 by Malindi Municipality in collaboration with research institutions and non-governmental organizations. The UMCP is divided into grid cells, each of which is 1 km^2 in size and manned by a cell scout or mosquito scout. These scouts are responsible for routine mosquito control and surveillance and report to the district public health offices within the Ministry of Health. The mosquito scouts working in this community-based LSM programme are responsible for creating awareness and conducting neighbourhood campaigns and house-to-house visits in order to educate the community about the intervention (*37*).

1.3 Role of the local authority

The local government should be well-informed about LSM, so that those in charge are in a position to judge the likely pros and cons of LSM locally. LSM should start with educating the community about LSM, so that people can take responsibility for environmental management around their homes. The support of the local authority is therefore indispensable to setting up education and awareness campaigns through schools, special meetings and local media. The local authority will also be essential in coordinating different sectors. As examples, the department for transport and public works can be encouraged to take some responsibility for malaria control by building roads that do not collect standing water either side, and the department for agriculture can help modify farming practices to avoid creating larval habitats, such as introduction of intermittent irrigation. An example of effective intersectoral coordination is the Malaria Free Initiative in Khartoum State, Sudan (**Annex 9**), which is run by the Ministry of Health in collaboration with the Public Works Department (to repair broken water pipes which are an important vector larval habitat), Farmers' Union and the Ministry of Agriculture (to promote intermittent irrigation), Ministry of Education (to involve schoolchildren directly in LSM), and the media (to increase radio and television broadcasts to raise public awareness and support for the campaign).

1.4 Role of the local population

The local community should be educated and involved in LSM as much as possible, for the following reasons:

- Local residents have the best knowledge of local geography;
- Workers must be allowed to enter private properties;
- Local residents can help with LSM by removing larval habitats around the home;
- Costs will be lower if modestly-paid members of the community conduct LSM.

This can be achieved as follows:

- The public (both schoolchildren and the wider community) should be educated about malaria transmission and the LSM programme;
- The programme should target all mosquito species, so as to not only control malaria and other vector borne diseases, but also to reduce nuisance biting;
- Local customs should be considered when planning the programme.

1.5 Political support

Securing and maintaining political support for a LSM programme is crucial for the following reasons:

- The government will be more likely to provide any additional funding required by the malaria control programme for adding LSM.
- Collaboration between the ministry of health and other sectors is desirable.
- New legislation for the compulsory removal of certain larval habitats or for preventing the creation of new larval habitats can be helpful.

1.6 Training field staff

Mosquito control officers can be trained by accompanying mosquito surveillance staff for one month during baseline data collection. Field staff should be trained in mapping and characterizing larval habitats, in searching for and identifying mosquito larvae and pupae, and in the LSM method of choice in the area. Since mosquito control officers are trained outside the main transmission season when many larval habitats will be dry, it is important that all larval habitats, both new and old, are found and treated during the wet seasons. Staff involved in larviciding should be trained in the Standard Operating Procedures for larviciding (**Annex 5**).

2. Building the evidence base for specific interventions

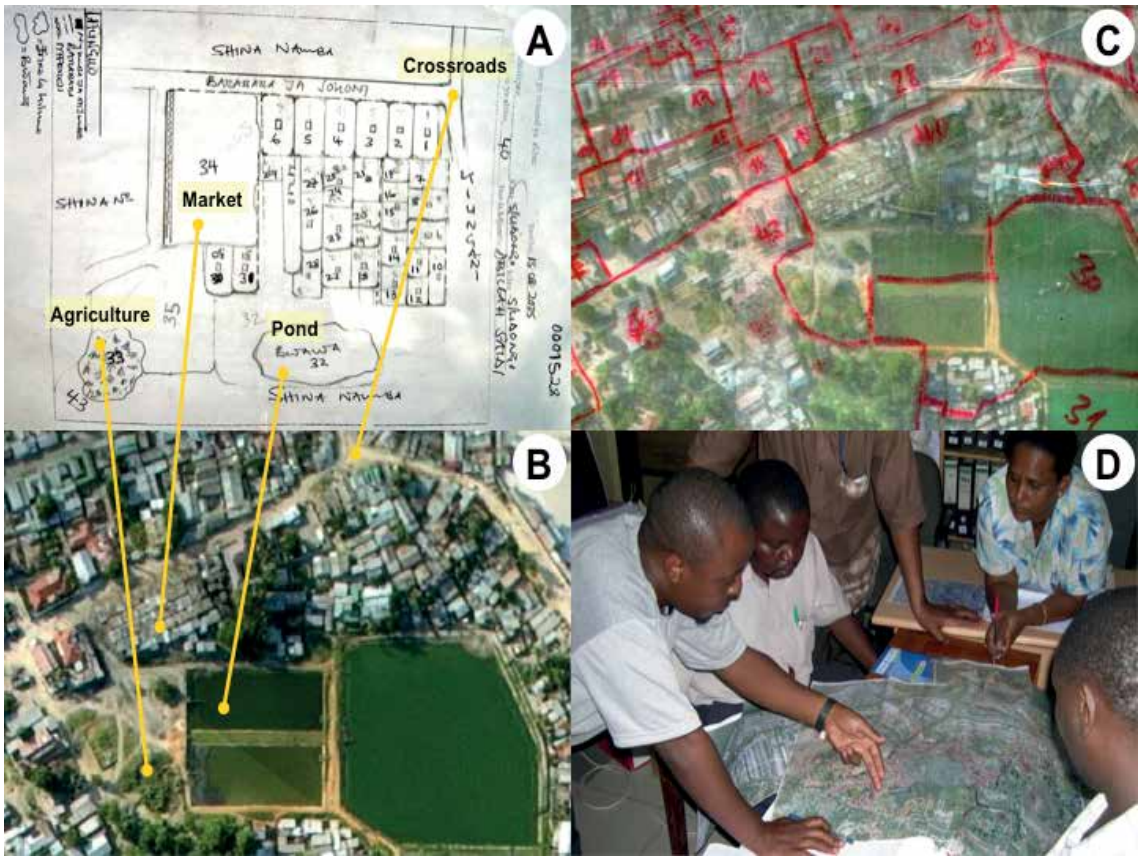
2.1 Baseline mapping of larval habitats and water bodies

Maps of all potential larval habitats must be created and updated at regular intervals, because some larval habitats may be temporary *(22)*. The following steps have been recommended for baseline mapping (*22*):

1. Map all boundaries of target areas

Maps of the target area should be designed to be useful to both management and field staff. Maps can be created simply, by members of the community and without electronic devices. Laminated aerial photographs (if available) should be taken into the field and used to sketch maps by hand. These can then be digitized using Geographical Information Systems (**Fig. 2**). Alternatively, breeding sites can be logged with high-precision hand-held GPS devices and maps created using computer software. These mapping techniques ensure that non-residential or industrial areas that may

Figure 2. Example of (A) sketch map, (B) aerial photograph, (C) field map and (D) programme planning from the Dar es Salaam UMCP (*22*)



initially be missed can be identified and mapped. Good maps of the target area allow work to be shared equally between field staff.

2. Divide target area into small plots of land

The target area should be divided into small plots of land, each of which is assigned to a larval surveillance officer for weekly larval surveillance (size of target area and plots depends on the terrain and the total number of habitats to be visited. The target area size can vary from 0.5–5 km^2 per week). Plots must be small enough to allow unambiguous description of all habitats in the plot, which can be easily identified by supervisors. Each plot can be described using a plot description form.

3. Map all potential larval habitats

Each potential larval habitat found should be identified and documented using a larval habitat surveillance form (**Annex 4**), in conjunction with a plot description form and the area map. When a larval habitat is first identified, it should be assigned a unique number, which is retained even if it becomes dry.

During the mapping process each larval habitat should be described by habitat type, size (habitat perimeter <10m/10–100 m/>100 m) when water is at largest extension and vegetation cover, since these can impact on the choice of intervention and/or larvicide. When a habitat is revisited regularly, it is useful to note whether the habitat has changed from the last visit, if it is dry or wet on the date of visit, the type of plants present (none/short/long/floating) and the depth of water (shallow/deep) (*22*).

When deciding on the categories for larval habitat 'type', these should be tailored to local vector ecology. In Dar es Salaam, larval habitats are classified in the following 12 types (*22*): puddles, tyre tracks; swampy areas; mangrove swamps, saltwater marshes; drains, ditches; construction pits, foundations, man-made holes; water storage containers; rice paddies; ridge and furrow agriculture; habitats associated with other agriculture; streams, river beds; ponds; others.

2.2 Baseline larval and adult surveillance

Baseline data on larval and adult populations should be collected, preferably for one year or one transmission season (if there is one main transmission season) or at least the major transmission season (if there are several). This allows major larval habitats and vector species to be identified and LSM to be well planned and targeted. It also facilitates evaluation of the impact of the intervention. Details on conducting larval and adult surveys are described in **Annexes 2 and 3**.

2.3 Monitoring and evaluation

Monitoring and evaluation (M&E) is integral to an operational vector control programme, in order to document progress towards achievement of goals and for data-driven decision-making at all levels of the programme. If the LSM programme is based on larviciding it is necessary to monitor whether: (i) the larvicide works (i.e. larvae are dead after 24 hours), (ii) larval control personnel are in the field applying larvicide according to their schedule, and (iii) adult vector densities are significantly reduced compared to baseline and are kept at a low level throughout the year.

1. Quality control of the product: Does the larvicide kill mosquitoes?

Only WHOPES-approved larvicides should be used for LSM, as listed in **Table 5** and at: http://www.who.int/whopes/Mosquito_Larvicides_Sept_2012.pdf. The list is updated as necessary and published on the WHOPES website.

With microbial larvicides it is important to be aware that for Bti only strain ***AM65-52*** and for Bs, only strain ***AM65-52***, are recommended. Not all larvicides are equally effective and they can vary in their stability, their potency and how they persist in the water column where they are available to be ingested by the target mosquito (anophelines are surface feeders). It is essential that there be quality control of all insecticides used in vector control, particularly bacterial larvicides, and especially those which have not gone through WHOPES testing.

WHO guidelines for the laboratory and field testing of mosquito larvicides (4) provide comprehensive guidance on how to carry out laboratory studies and small-scale and large-scale field trials to determine the efficacy, field application rates and operational feasibility and acceptability of a mosquito larvicide.

If a control programme lacks a laboratory, field trials of formulated products can be performed on a small scale against target mosquitoes, preferably in representative natural breeding sites or, where such trials are not feasible, under simulated field conditions. For instance, for testing Bti against *An. gambiae* s.l. large plastic bowls full of water, with a little soil added, provide ideal breeding sites for wild mosquitoes (*81*). After a few days wild mosquitoes will have laid eggs in the bowls and wild *An. gambiae* s.l. larvae will be abundant. Different concentrations of larvicide are then added to the bowls, with some left untreated to serve as controls. At daily intervals the number of anopheline larvae can be checked to see how effective the larvicide is relative to the untreated bowls. It is important to make these assessments independently from those selling the larvicides so that the programme manager can be confident that the larvicide works well under local conditions.

2. Quality Assurance of the implementation: checking field application of larvicides

Larviciding is a 'learning activity'. Larviciding applicators learn where the local mosquito breeding sites are and improve their effectiveness from one round to the next. Nonetheless it is essential

that the control manager has full confidence in the applicators. For this reason it is important to have an independent group of assessors who check the quality of the larviciding programme, as exemplified in Dar es Salaam (*22,82,83*), where an observer follows larvicide applicators doing their daily work. They report on whether the applicators followed their schedule correctly, whether they entered all the stipulated areas, how habitats were searched for larvae, and how they interacted with residents. This information can then be fed back to the programme manager.

Random larval spot checks to monitor the larviciding applicator's performance should be carried out in treated areas throughout the malaria transmission season(s) to determine the proportion of habitats containing early and late instar larvae. Ideally 30–40 habitats within every applicator's area should be checked at least once per month. Inspection of sites should take place 1–2 days after the habitats were treated with larvicide. The results from this spot checking will inform the programme manager of the performance of the applicators. In addition sentinel sites should be checked in an untreated area outside the treated area to monitor the seasonal fluctuations in mosquito numbers. For a detailed description of such a programme, see (*84*).

3. Monitoring LSM impact on adult vector populations and malaria transmission

It is essential to monitor the impact of larviciding on malaria transmission. Discerning epidemiological impact on malaria incidence is often difficult when there are other on-going control measures, including improved diagnosis and treatment, the use of LLINs and implementation of IRS. A more proximate measure is to monitor the impact of the LSM programme on adult mosquito populations in the target area (*83,84*). This will show first and foremost whether the LSM programme has been able to locate and treat all of the larval habitats in the target area, or if there were additional habitats unseen or missed that still contribute to adult vector populations and malaria transmission. The simplest way to do this is by collecting adult mosquitoes indoors, using CDC light traps operating next to individuals sleeping under a LLIN, human-landing catches or pyrethrum spray knock down catches of indoor resting mosquitoes in the early morning. In order to reduce the risk of spill-over, where mosquitoes from untreated areas fly into treated areas, it is important to establish a network of about 30 houses in the centre of the treated area. These houses should be sampled weekly during the transmission season. If possible, it would also be beneficial to sample from a similar number of houses in an untreated area nearby to determine the reduction in mosquito densities achieved by LSM. Preferably all houses should be randomly selected so that the average intensity of transmission in the area is captured.

The entomological measurements can be linked to the incidence of malaria if health records are routinely collected from health centres within and outside the treated areas. It should be stressed that only malaria cases confirmed by microscopy or by rapid diagnostic tests should be measured. In most situations where LSM is being implemented there are also improvements in malaria diagnosis and treatment as well as improvements in other vector control measures, especially improved use of LLINs and in some situations, IRS applications. This can make it difficult to attribute changes in malaria incidence or prevalence in the community directly to LSM. Therefore, it is essential to have entomological indicators, such as the LSM impact on adult vector populations, to determine whether the LSM is contributing to malaria control.

Detailed assessments of the effectiveness of LSM are best obtained by carrying out cluster randomized controlled trials. Further technical support on how such sampling frameworks should be established can be provided through WHO. While rigorous M&E requires financial and technical resources – including epidemiological expertise as well as entomological expertise – this is essential to confirm that the investments in LSM are providing a sufficient return in epidemiological impact, or whether those funds should be used for other malaria control efforts.

In conclusion, both spot check larval habitat surveillance for monitoring product efficiency and staff performance, and adult mosquito collections for monitoring LSM impact need to be included in LSM programmes. M&E strategies differ in different ecological settings due to the differences in vector ecology, scale of the programmes and programme design. The impact of the intervention

on the disease can be assessed through government health records in conjunction with the monitoring of other disease control efforts.

2.4 Establishing a database

Larval and adult surveillance forms should be collated by supervisors and a database established in a computer programme such as Microsoft Office Access.

3. Implementing LSM

Full details on factors to consider in deciding which LSM interventions (if any) are appropriate are in **Chapter 1** and details on practical implementation of LSM are provided in **Chapter 3** and **Annex 7**.

LSM must be tailored to the local setting. If sectors outside the health sector are to be involved in LSM, the responsibilities for different larval control activities will need to be planned and shared accordingly. Wherever possible, priority should be given to environmental management to reduce larval sources.

Where environmental management is not possible, larviciding can be conducted provided that baseline data on larval habitats has been collected and an M&E system has been set up. It may be preferable to begin larviciding initially in a small pilot area before expanding to the full target area, as was done in Dar es Salaam, where three wards were initially selected for larviciding based on the capabilities of staff, and more wards were added later on (*22,34*). This has two advantages: (i) it allows the infrastructure for larviciding to be built up gradually and any problems to be resolved efficiently and (ii) continued surveillance in non-intervention areas allows more accurate assessment of the effectiveness of larviciding when it is first implemented.

Larviciding should be conducted by separate teams from those responsible for larval surveillance (**Fig. 1**). For example, each larval control officer could be given responsibility for larviciding in one plot. Supervisors should provide the mosquito control officers with a timetable for plots to visit each day and the entire area should be searched for any site containing water. All larval habitats should be targeted and treated if wet at the time of visit, irrespective of the presence or absence of larvae, and visited every week (*22*).

4. Data collection and operational reporting

4.1 Reporting on habitats modified, manipulated or treated

A good system of reporting which habitats have been modified, manipulated or treated is essential. Daily insecticide use by mosquito control officers should also be recorded and reported to supervisors. To do so, mosquito control officers should record the following information in a standard form (**Annex 5**): date; plot visited for LSM; total weight of larvicide received per day; total weight of larvicide remaining at end of day; calculated weight of larvicide used per day. One standard form should be completed for every day on which larval control is conducted.

Supervisors can monitor larvicide use using information collected in these forms. Larvicide use can also be cross-checked at both local and central stock facility. This allows new stock to be ordered and shipped in good time (*22*).

4.2 Larval and adult surveys

Both larval and adult surveillance must be undertaken continuously to monitor the impact of larval control efforts and to help direct future efforts; larval surveys alone, without adult vector monitoring, are not sufficient. This can be achieved by adapting the systems used for baseline entomological data collection into spot checks.

Data on larval presence (collected in larval surveillance forms (**Annex 4**) by larval surveillance officers should be collated and interpreted at local level, using weekly summary forms (**Annex 6**).

This allows larvicide to be rapidly reapplied where necessary and is a simple system that does not require a computer. Within 24 hours, supervisors should read and respond to all larval surveillance data. All larval surveillance forms should be collated in pre-labelled folders with the supervisor's summary sheet. These folders are then passed to the LSM programme manager, who should check that all forms have been correctly filled in, recording the results of this in a checklist. The totals from the bottom of each ward supervisor's summary form should be entered into an Excel or Access spreadsheet which automatically generates summary statistics, tables and charts (*22*) (**Fig. 3**). These can then be circulated to the relevant managers each month and flaws in the system can be identified.

It is very important to record early stage larvae (1st and 2nd instars) and late stage larvae (3rd and 4th instars) and pupae separately. An abundance of late stages, or presence of pupae, can indicate LSM failure.

Figure 3. Example of summary data table from the Dar es Salaam UMCP (*22*)

Municipal larval survey – Monthly summary report																	Month:										Year:									
Area	Number of 10-cell units	Total habitats					Wet habitats					Habitats with early instar Anopheles					Habitats with late instar Anopheles					Habitats with early instar Culicines					Habitats with later instar Culicines					Habitats with pupae				
Week:		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
BUGURUNI WARD																																			
Mnyamani	82																																		
Malapa	66																																		
Madenge	49																																		
Kisiwani	91																																		
Totals	288																																		
Proportion of wet habitats occupied:																																			
ILALA WARD																																			
Sharif Shamba	43																																		
Mafuriko	35																																		
Karume	33																																		
Kasulu	39																																		
Totals	150																																		
Proportion of wet habitats occupied:																																			

CHAPTER 3
Conducting LSM

This chapter, with **Annex 7**, describes in detail how to implement the four types of larval source management: habitat modification, habitat manipulation, larviciding, and biological control.

1. Habitat modification

Habitat modification is a permanent alteration to the environment, defined as 'a form of environmental management consisting of any physical transformation that is permanent or long-lasting of land, water and vegetation, aimed at preventing, eliminating or reducing the habitats of vectors without causing unduly adverse effects on the quality of the human environment' (*7*).

Habitat modification can be a key component of LSM and historically, before the advent of pesticides, was the major method of malaria vector control. The engineering techniques for drainage and surface water management have been well developed and documented over the past century. More detail on the techniques of habitat modification is provided in **Annex 7**: this is usually not a core function of the NMCP but rather other sectors such as the public works departments, ministries of local government and housing, etc.

It is important that the ministry of health constitute a national Integrated Vector Management Committee that includes representatives from the NMCP as well as from the ministry of agriculture, ministry of the environment, and other ministries and municipal agencies which may be involved with surface water management, road building, urban drainage, housing construction etc. Through the National IVM committee the NMCP should ensure that the principles and techniques for LSM, especially habitat modification, are known and adopted by other relevant agencies and departments involved in IVM efforts.

2. Habitat manipulation

Habitat manipulation is a form of environmental management aimed at producing temporary conditions that are unfavourable to breeding of vectors. Unlike habitat modification, habitat manipulation must be repeated to remain efficacious, and is normally directed at one particular vector species.

The following methods of habitat manipulation are described in **Annex 7**: controlling water levels (including intermittent irrigation), stream flushing, shading, clearing of aquatic vegetation, straightening and steepening of shorelines, changes to water salinity and water pollution.

3. Larviciding

Larviciding is complementary to environmental management. This section outlines how to select a suitable larvicide and how best to apply the correct dosage and appropriate formulation.

3.1 Selection and application of larvicides

WHOPES-recommended compounds, formulations and dosages for control of mosquito larvae are listed in **Table 5**. When selecting larvicides and specific formulations, those involving the least hazard to humans and the environment are preferable. Other considerations are total programme costs, transportation requirements, availability of suitable equipment and most importantly, storage requirements and shelf life. Costs should be calculated 'per person protected per day or per year', not simply on purchase price per kilogram.

In addition, tests should be conducted to determine the susceptibility of local vectors to candidate formulations. To determine the *minimum effective dosage* for a larvicide, laboratory bioassays should be conducted. To determine the *optimum effective dosage*, field trials in natural or artificial habitats should be conducted following WHO guidelines for testing mosquito larvicides (*4*).

Formulations

Most larvicides are available in a variety of formulations (*85,86*), including:

- *Emulsifiable concentrate (EC):* Solution of an insecticide in a solvent; a liquid, homogeneous formulation to be applied as an emulsion after dilution in water. Application is through pouring or spraying over water surface, most often using a portable sprayer. ECs are the most commonly used formulations for organophosphates.
- *Suspension concentrate (SC):* A formulation obtained by suspending solid water-insoluble pesticides in water to produce a flowable liquid product. It can be applied undiluted or diluted with water depending on active ingredient/formulation and application needs.
- *Water Dispersible Granule (WG):* A formulation consisting of granules to be applied after disintegration and dispersion in water. This formulation provides the storage stability of a dry product with the application versatility of a liquid spray (note: in some cases, WG formulations can be applied directly to artificial/natural containers for control of mosquito larvae).
- *Wettable powder (WP):* Dry powder of the insecticide formulated with wetting (dispersing) agent, which promotes rapid mixing with water to form a suspension.
- *Granules (GR, GG, FG, MG, CG):* A free-flowing solid formulation of a defined granule size range ready for use. Granule formulations allow for direct penetration of dense aquatic vegetation better than with liquid formulations. Application is typically made with portable blowers or by hand. Special forms of granule are as follows:
  - GG (macrogranule): A granule in the particle size range from 2000 to 6000 microns
  - FG (fine granule): A granule in the particle size range from 300 to 2500 microns
  - MG (microgranule): A granule in the particle size range from 100 to 600 microns
  - CG (encapsulated granule): A granule with a protective or release-controlling coating.
- *Pellets:* A formulation obtained by extrusion of inert materials impregnated with active ingredient. Like granules, these formulations allow for direct penetration of dense aquatic vegetation better than with liquid formulations. Typically, pellets allow for more controlled release of the insecticide, thus extending the residual activity of the product.
- *Briquettes (BR):* Blocks of an inert material impregnated with insecticide. Depending on the inert composition, briquettes may either float or sink. Degradation of briquettes and the inert composition allows for controlled release of the insecticide. This type of formulation is typically applied by hand.

The choice of formulation depends on a number of factors including but not limited to:

- Regulatory or WHOPES requirements/specifications
- Larval habitat size
- Extent and density of vegetation

- Cleanliness of the water
- Desired residual
- Worker safety
- Storage stability requirements
- Application flexibility of formulation
- Potency and/or concentration
- Ease of use
- Equipment available.

Unless larval habitats are very extensive (e.g. river flood plains), most larvicides can be applied by ground applicators utilizing either ground equipment (boat, vehicle mounted dry product spreader/liquid sprayer, handheld or backpack dry product spreader/liquid sprayer) or by hand for specific circumstances. Typically, liquids can be applied in dilute or concentrated form. Dilute mixtures are usually used for application of large volumes using large droplets. Low volume (LV) and ultra low volume (ULV) application (of fine sprays, mists or aerosols) can be used for more concentrated mixtures. This technique applies the minimum amount of liquid (<5 L/ha) and if conducted correctly, results in substantial savings through its speed of application, lower handling costs and smaller staff requirements. In addition, coverage of large surface areas can be achieved by taking advantage of wind drift under appropriate atmospheric conditions.

Dosage

The dosage is usually expressed as volume or weight of active ingredient applied per unit surface area or volume of the larval habitat. It is determined by the concentration or potency (as in the case of bacterial larvicides) of the formulation. It is very important to calibrate the rate of emission, swath width and speed of application to ensure the correct dosage rate is applied. Under operational conditions it is not feasible to measure the volume of each habitat before applying a given dosage of larvicide, therefore calibration will be implemented based on material requirement per surface area (e.g. g/m^2) (*78*).

Equipment

The choice of equipment, equipment calibration, and applicator training are critical for the success of LSM operations. The choice of equipment will depend on a variety of factors:

- Choice of larvicide formulation (liquid or granular)
- Nature and size of targeted larval habitats
- Availability of fuel, oil and maintenance services for power equipment
- Local capacity to securely store equipment that may be useful for agriculture or other pursuits.

While hand application of granules may be sufficient for many applications, such as routine treatment of small urban sources, many LSM operations will require the use of some equipment. This will certainly be true when liquid sprays are employed and for application of granules in large areas.

For further details on appropriate equipment for applying larvicides, see (*87,88*).

Application of liquid sprays

Liquid larvicide sprays are used in a number of applications due to their efficiency and potential cost savings. High volume or low volume sprays can be selected depending on available equipment, type of habitats and specific application objectives. The availability of clean water for the preparation of sprays can be a limiting factor in some locations. Extensive, dense, emergent vegetation in larval habitats can limit the effectiveness of liquid sprays.

Manual application of high volume sprays is an appropriate choice for small to medium sized habitats that have a low density of emergent vegetation. Manual equipment requires no fuel and minimal maintenance, so it can be carried out in areas where these resources are limited. For high volume, hand application of liquid insecticides to larval habitats, compression or backpack sprayers (**Fig. 4**) fitted with a solid stream nozzle (**Fig. 5**) should be employed using the swathing spray (**Figs. 6–8**) or spot spray methods (**Fig. 9**).

Figure 4. Examples of suitable spray equipment for high volume, hand application of liquid larvicides

Figure 5. Examples of solid stream nozzle configurations

Disk – no core

Solid stream

Figure 6. Schematic of swathing spray method
Swing spray wand back and forth to create an arc while walking through the source. Always make a full semi-circle arc (180°) and keep the wand pointed high. Spray mix rate will depend on effective swath, walking speed, and sprayer flow rate.

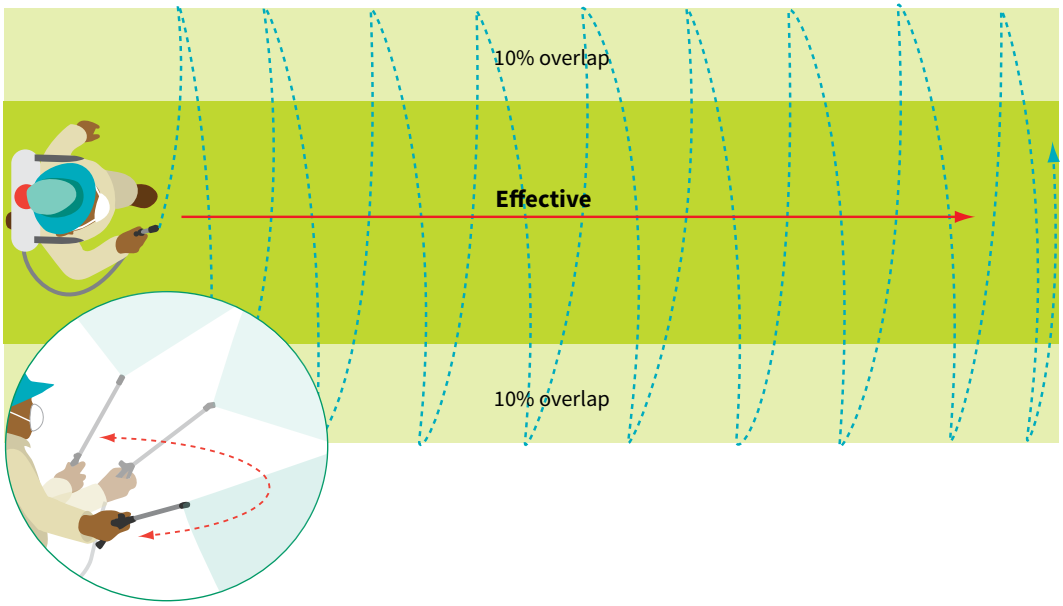


Figure 7. Application of swathing spray to a large larval source

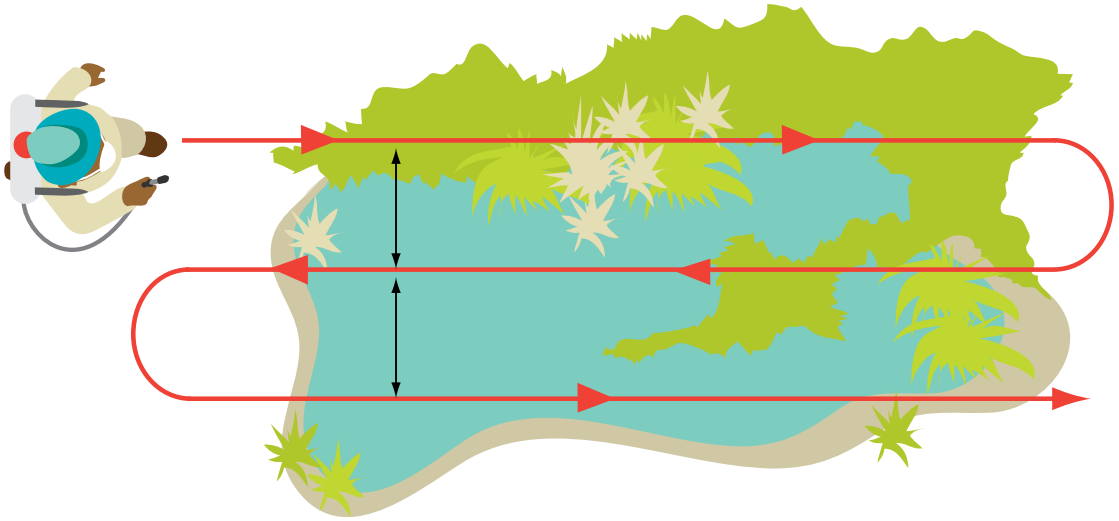
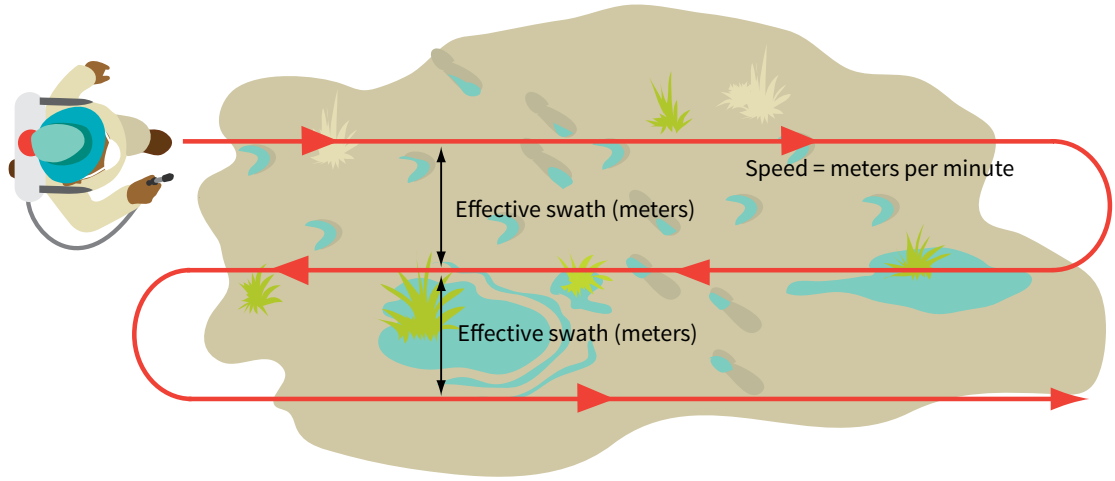


Figure 8. Application of swathing spray to a concentration of small larval sources



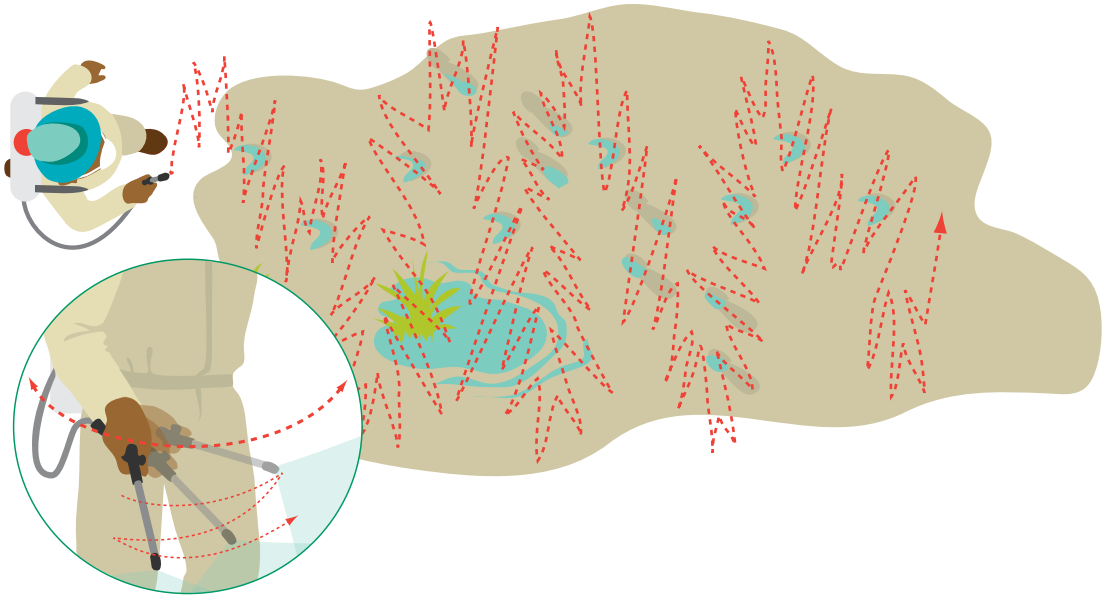
Swathing spray method

The swathing spray method takes advantage of a projected spray stream and oscillation of the spray wand by the applicator. This allows treatment swaths of up to 10m wide (with 180° sweep) with this equipment and is generally used for larval sources >10 m^2 in size or areas containing multiple small sources (puddles, residual streambed pools, and hoofprints) in close proximity to each other. Calibration of the swathing spray is based on measured swath width, walking speed, and sprayer flow rate. Generally, larvicide concentration in the spray mix is selected based on calculated spray volume per surface area achieved (L/ha).

The calibration of manual, large volume swathing application of liquid sprays is based on:

- **Speed** of travel (m/min)
- Width of **effective swath** (meters wide)
- **Flow** rate of sprayer (L/min)
- **Concentration** of product (g/L).

Figure 9. Schematic of spot spray method for individual larval sources less than 10 m² in size
Note that the spray wand is oscillated to cover the surface evenly with drops from a solid stream.



Spot spray method

The spot spray method is used to treat individual small sources rapidly (**Fig. 9**). Oscillation of the spray wand achieves even coverage of the source, but is more directed to cover specific small sources and avoids waste of larvicide.

The calibration of manual, spot application of liquid sprays is based on

- **Time** to spray (m^2/min)
- **Flow** rate of sprayer (L/min)
- **Concentration** of product (g/L).

Often, it is most efficient to calibrate sprayers for both swathing spray and spot treatments, since both methods will need to be employed during the course of daily treatments. In this case, spray concentration should be selected based on calibration for swathing spray, and applicators should be trained to mentally 'time' their spot treatments so as to deliver sufficient material in spot treatments. For example, if a sprayer has been calibrated to deliver 20 L of total spray volume per ha with walking speed of 60 m/min and a 10 m swath, then the applicator is treating 600 m^2/min (10 m^2 per second). In this case, the applicator should be trained to spray for <0.1 sec/m^2. This can take some practice, but is usually quickly mastered by applicators.

Low volume (LV) and ultra low volume (ULV) spray methods

For low volume sprays of mosquito larvicides, power backpack and vehicle-mounted mist blowers or ULV sprayers can be utilized to disperse fine (small drop) sprays to target cryptic habitats and cover large areas (**Figs. 10–12**). With proper atmospheric conditions and spray strategies, large open habitats such as rice fields can be covered rapidly. These methods can also be used for treatment of multiple small sites such as ground pools or animal hoof prints covering large surfaces, or larval habitats that exist within fenced compounds.

Truck-mounted ULV and backpack LV spraying of Bti AM65-52 WG was successfully used in a malaria elimination project on Tekong Island, Singapore (*89*). These methods have also been successfully used for control of container Aedes spp.

Figure 10. Low volume spraying of Bti AM65-52 WG for malaria vector control on Tekong Island

Figure 11. Truck-mounted low volume spray equipment under test for coverage of wide areas

Figure 12. Truck-mounted ultra-low volume sprayer in operational use

Granule and Pellet Application

Solid formulations including granules and pellets are often required for penetration of emergent vegetation in larval sources. They are applied undiluted, and can be applied by hand without equipment. Equipment including manual rotary disk spreaders (**Fig. 13**), power backpack blowers (**Fig. 14**), vehicle-mounted rotary disk spreaders and blowers (**Fig. 15**) are useful to improve swath coverage, application efficiency and homogeneity of coverage. Hand-held spreaders typically deliver swath widths of 5–10 m. Calibration is based on measured swath, walking speed, and desired application rate.

Figure 13. Hand held rotary disk equipment for granule application

When using power backpack blowers (**Fig. 14**), the swathing spray method described above for liquid sprays is also applied. Swaths of 2030m can often be achieved depending on formulation and equipment. This enables rapid coverage of large larval sources when teams of two applicators share responsibility for material transport and application.

Calibration of equipment flow rate based on swath width, walking speed and desired application rate is essential for both efficacy and efficiency.

Figure 14. Power backpack suitable for granule and pellet application

Figure 15. Example of vehicle-mounted rotary spreader

Care of equipment

Equipment design and maintenance should conform to WHO specifications. Equipment should be tested before storage or use, before being cleaned and dried, and a stock record made of its availability along with any spare parts required in the field. Field supervisors should ensure that equipment is properly dealt with following a day's operation, prior to night storage or transportation (*5*), including:

- Removal and disposal of residual larvicide (except in the case of granular formulations);
- Flushing of liquid application equipment at least three times with clean water so that all parts that come into contact with pesticide are cleaned. Rinse water can be kept for use in diluting larvicides;
- Careful inspection of equipment and reporting of damage;
- Each team of field staff should be supplied with the necessary tools for repair of equipment and spare parts. A few members of staff in the area should be trained in the repair of equipment.

Safety considerations

When working with larvicides toxic to humans, hand and backpack sprayers should not be filled more than three-quarters full. To protect operators from contaminations while carrying sprayers with larvicides toxic to humans on their shoulders, a piece of impermeable material should be placed between the sprayer and his or her overalls, to be washed when contaminated (*5*).

Staff should be well-trained in the use of equipment. Larvicides and equipment should be safely handled. Overalls, shoes and hat should be sufficiently protective for handling diluted larvicides. When preparing and diluting concentrates, an impermeable apron, rubber boots, gloves and a face mask should be worn. Pesticides should be washed off clothing as soon as possible. Bare hands should never be used to mix pesticides, even those with the lowest toxicity (*5*).

Frequency and timing of application

The timing of application must be tailored locally since it is determined by the local species, seasonality of transmission, precipitation, exposure to the sun, water quality, residual efficacy of the larvicide, mode of action of the active ingredient, and type of larval habitat. The supplier of the chosen larvicide will be able to provide more specifics regarding frequency and timing of applications. In many cases, manufacturers will work with a control programme to optimize application to meet programme needs and public health requirements. In addition, this information will be adjusted during the piloting phase of the programme before large scale applications.

Storage and distribution of larvicides (*90,91,92*)

Larvicides should be stored in a secure, central location and the necessary quantities distributed to local stores or offices weekly for larvicides that require a weekly application. Records of the amount delivered and taken away should be kept at both central and local stores. Stores should be dry. If a specially dedicated store is not available, locked cabinets may be used (*5*). Storage requirements such as temperature and shelf-life need to be considered when choosing a specific larvicide formulation, e.g. liquid formulations of bacterial larvicides have a shorter shelf-life under tropical conditions. In areas with high average temperatures, dry formulations that can be mixed with water on demand (e.g. water dispersible granules) or granular formulations are preferable as they typically have a longer shelf-life relative to liquid products. Another potential advantage of dry products is that they require less storage space in some cases, and shipment costs are reduced as their potency may be higher per unit of volume.

3.2 Types of larvicide

For detailed information and WHO specifications for larvicides, see WHO specifications for pesticides used in public health: http://www.who.int/whopes/quality/newspecif/en/

Oils and surface agents

Oils kill larvae quickly by specific toxicity or suffocation but usually remain effective for only a few hours or days. There are many grades of oil suitable for larval control. More volatile oils tend to be more toxic but less residual since they are more prone to break into globules or to being blown by wind to margins of the pool. Specifically prepared oils are available which contain surface-active agents that increase spreading power and toxic action. There are no formal recommendations from WHO on the use of oils as surface agents for larval control.

Synthetic organic compounds

Chlorpyrifos

Chlorpyrifos is *not* currently recommended by WHO for the control of malaria vector larvae (*71*). It is an organophosphorus compound generally used for moderately to highly polluted water (such as catch basins, ditches containing sewage, pit latrines, cesspits, etc.), where its residual effect lasts for several weeks. The WHO hazard rating is moderately hazardous; it is moderately toxic to mammals and birds and highly toxic to fish and therefore should never be used in drinking water or in water containing fish.

Fenthion

Fenthion is an organophosphorus compound with a long residual effect. It is generally used to treat polluted water in ditches, ponds, swamps, septic tanks and other larval habitats that are not sources of drinking water. It is more effective than temephos in polluted water.

- *Toxicity*

  WHO hazard rating: moderately hazardous
  Fenthion is highly toxic to mosquito larvae, which it rapidly kills. It has high toxicity to humans, mammals and birds, and therefore safety precautions should be taken. At the recommended doses, fish are not affected.

- *Formulations*

  Fenthion is usually procured as emulsifiable concentrates (46% and 84.5% w/v active ingredient) and sand granules (2% active ingredient).

- *Preparation and use*

  Large surfaces: the recommended dosage is 22–112g active ingredient per ha and the final concentration in treated water should not exceed 0.1mg/l (*71*). Emulsifiable concentrates are applied directly or after mixing with water. Sand granules (2%) are applied using a portable blower at 5.5kg/ha and used in shallow water and slow moving streams shallower than 30 cm. Granules are more suitable for treating areas with dense vegetation or debris.

Malathion

Malathion is an organophosphorus compound which is not currently recommended by WHO for the control of malaria vector larvae (*71*). It is highly toxic to insects but at recommended dosages it is safe for humans and domestic animals, though it may cause some harm to fish. It is primarily used for IRS and is applied by specialized mosquito control agencies only. It is not routinely used for larval control.

Pirimiphos methyl

Pirimiphos methyl is an organophosphorus compound, effective against many insects including larvae, with levels of effectiveness similar to fenthion. It is relatively unstable in polluted water.

- *Toxicity*

  WHO hazard rating: slightly hazardous
  Pirimiphos methyl is highly toxic to larvae. It is much less toxic to humans than fenthion but should not be used to treat drinking water.

- *Formulations*

  Pirimiphos methyl is available as a 50% emulsifiable concentrate.

- *Application*

  Large surfaces: The dosage should be 50–500g active ingredient per ha (*71*). It is applied using hand compression sprayers. This treatment remains effective for 1–11 weeks, depending on water quality.

  Container breeding: The dosage should be 1mg/l (*71*).

Temephos

Temephos is an organophosphorus compound. It is a brown, viscous liquid, which is stable at ambient temperature and effective in clean, moderately and heavily polluted waters. It is relatively safe and a low dose is sufficient.

- *Toxicity*

  WHO hazard rating: unlikely to pose an acute hazard in normal use
  Very low toxicity to fish, birds, mammals and other non-target organisms. Recommended for application to drinking water.

- *Formulations*

  Commonly available as EC (46% and 20% w/v/ active ingredient) and granules (1% active ingredient).

- *Preparation and use*

  Large surfaces: the dosage of active ingredient should be 56–112g/ha (*71*). 56 g active ingredient per ha is recommended for clean, open water and 112g/ha if there is dense aquatic vegetation. Granules are more effective where there is dense aquatic vegetation and should be applied every 1 to 3 months. Granules and water suspensions of the EC are applied by spraying.

  Container breeding: the dosage for drinking water should be 1mg/L or 20g (2 teaspoonfuls) of a 1% sand granule formulation in a 200L drum (*71*). Liquid formulations are applied by pouring directly onto the water surface. Granules can be added directly to drinking water and will be effective for 5 weeks. Floating temephos-impregnated plastic pellets are also available; these last for 6 weeks and are suitable for anopheline control as the larvae feed at the surface.

Pyrethroids

Pyrethroids (e.g. deltamethrin, permethrin) are not recommended by WHO for the control of malaria vector larvae, as they harm all insects, fish, crustaceans and other aquatic animals. They may also select for pyrethroid resistance in the adult mosquito population.

Bacterial larvicides

Bacterial larvicides (BL) have a number of advantages. They are highly efficacious against malaria vectors, do not harm non-target organisms, are selective in action, are unlikely to produce resistance and are safe for humans to handle (*22*). Bacterial larvicides differ from conventional chemicals or biochemicals in that identical strains produced under different conditions by different manufacturers will have varying end-use product quality and biological performance. It is important to use WHOPES-recommended bacterial larvicides and formulations that are linked to specific strain numbers to ensure biological and performance equivalency (*71*).

Bacillus thuringiensis *subsp.* israelensis (*Bti*)

Bti is a naturally occurring, spore-forming bacterium found in soil and aquatic environments throughout the world. During sporulation, Bti produces a highly specific delta endotoxin, which is only toxic to larvae of mosquitoes, black flies and closely related flies upon ingestion. Bti is effective where insects have developed resistance to synthetic and/or biochemical larvicides. Residual efficacy is dependent on target habitat/species complex and formulation type.

- *Toxicity*

  This bacterium produces insecticidal crystal proteins that kill susceptible larvae within 24h of ingestion. At recommended dosages it is harmless to non-target aquatic invertebrates, insects, fish, birds, animals and humans. It is safe for use in drinking water or on irrigated crops. Bti has not been issued a WHO hazard rating due to its perceived low risk.

- *Formulations*

  — Generally available as water dispersible granules, granules, suspension concentrates, wettable powders, and briquettes. Biological activity (biopotency) is expressed in International Toxic Units per milligram (ITU/mg).

— *Suspension Concentrates (SC):* Biopotency is typically 600 or 1200 ITU/mg. Used for a variety of habitat settings but are best used in open waters with minimal to no vegetation. Reapplication is typically at 7 to 14 days depending on programme objectives.

— *Water Dispersible Granules (WG):* Biopotency of WHOPES-recommended Bti strain AM65-52 WG is 3000 ITU/mg. This formulation can be applied as (i) a liquid spray to open habitats with little or no vegetation, (ii) an area-wide spray to control larvae in cryptic habitats and (iii) directly to artificial and natural containers. Residual efficacy depends on formulation quality, target habitat/species and a number of other abiotic/biotic factors. Reapplication can range from 7 to 14 days for surface water treatment for *Anopheles*, to three months or more for *Aedes* control in domestic water containers. In practice, retreatment should be done when 4th instar larvae reappear.

— *Wettable Powders (WP):* These powders come in a variety of biopotencies and must be mixed with water before application. Reapplication is typically 7 to 14 days.

— *Granules:* Bti granules come in a variety of carrier types and potencies with the primary purpose of delivering the maximum amount of Bti into the water. Biopotency of WHOPES-recommended Bti strain AM65-52 GR is 200 ITU/mg. Granules minimize drift for more targeted applications and are better at penetrating vegetation than liquid sprays. Reapplication is typically at 7 to 14 days.

— *Briquettes (BR):* Briquette biopotencies vary depending on the manufacturer and the technical powder used. These formulations release Bti for 30 to 180 days (residual depends on BR type and biopotency) and their effectiveness is not altered by alternate wetting and drying, making them appropriate for both permanent and temporary habitats. Briquettes can be ring-shaped (which usually float) or in brick form (which usually sink). These formulations are less effective for open water (wind may blow them to the margins), so applications are generally used to treat small domestic larval habitats such as ponds, basins and tanks. Briquettes become clogged in polluted water so should be used for relatively clean habitats.

- *Preparation and use*

— *Liquid Sprays:* Suspension Concentrates (SC), Water Dispersible Granules (WG) and Wettable Powders (WP) can all be used for liquid spray applications. The rates used will depend on formulation type, potency, vector species and habitat. SC formulations can be either applied diluted or undiluted, depending on the volume of water required to cover the habitat. WG formulations are typically mixed in water before spraying open habitats or applied directly to natural and artificial containers without pre-mixing in water. WP formulations require mixing in water for all target habitats. All formulations can be applied using hand compression pumps or other spray equipment. Recommended application rates for WHOPES recommended Bti WG, strain AM65-52 are:

Open habitats: 125–750g formulated product/ha (*71*)

Containers: 1–5mg formulated product/L (*71*).

— *Granules:* These can be hand applied or applied with portable blowers to habitats with or without vegetation. Recommended application rates for WHOPES recommended Bti GR, strain AM65-52 are:

Open habitats: the dosage should be 5–20 kg formulated product/ha (*71*).

— *Briquettes:* Floating ring formulations are applied by hand (1 unit/10m^2 surface area) and may need to be attached to plants or other fixed objects with string to avoid wind disturbance. Brick formulations typically sink to the bottom of the habitat and do not need to be secured. Rates for these BR forms are similar to the floating ring forms. Briquettes should be stored in sealed packages in a cool place to protect from humidity.

Bacillus sphaericus *(Bs)*

Bs is also a naturally occurring, spore forming bacterium found throughout the world in soil and aquatic environments. Bs is more target specific than Bti and can provide longer residual control than Bti in highly organic environments such as catch basins, sewage, waste lagoons, animal waste ponds or septic ditches. Possible explanations for the residual effects of Bs include recycling through germination, growth and new spore formation in the larval gut, non-target insect redistribution and the high buoyancy framework of the spore-crystal complex. There are currently no WHOPES-recommended strains of Bs for mosquito control. However, this bacterial larvicide has been used operationally in selected African settings.

- *Toxicity*

  Bs is more target-specific than Bti. Bs is highly toxic to *Anopheles* and *Culex* larvae but does not harm non-target organisms and is not effective against blackflies or *Aedes aegypti*.

- *Formulations*

  Available formulations include granules (GR, FG; typical label biopotency 50 BsITU/mg), Water Dispersible Granules (WG; typical label biopotency 650 BsITU/mg), Suspension Concentrates (SC), Briquettes (BR) and Granules in Water Soluble Pouches (WSP) for placement applications. A combined Bs and Bti formulation is also available which can be applied to all larval habitats.

- *Preparation and use*
  - *Liquid Sprays:* SC and WG can be used for liquid spray applications. The rates used will depend on formulation type, potency, vector species and habitat. SC can be either applied diluted or undiluted, depending on the volume of water required to cover the habitat. WG is typically mixed in water before spraying open habitats. For liquid applications, product should be thoroughly mixed and should be applied within 48–72h. Product that has been left to settle should be re-suspended prior to application. The product is effective for up to 28 days. Application rates depend on the target species and habitat and residual activity.
  - *Granules (GR, FG):* These formulations can be applied by hand or applied with portable blowers to habitats with or without vegetation. The product is effective for 21–35 days. Application rates range from 5–80 kg/ha and depend on the target species and habitat and residual activity.

Spinoyns

The product Spinosad is a combination of two metabolites extracted from the bacterium *Saccharopolyspora spinosa* via fermentation. Its mode of action is as a nicotinic acetylcholine receptor (nAchR) allosteric activator.

- *Toxicity*

  WHO hazard rating: unlikely to present acute hazard in normal use
  Spinosad has low acute toxicity to non-target organisms.

- *Formulations*

  Spinosad is available as suspension concentrates at 120 to 480 g spinosad/L, wettable powders, water dispersible granules, tablets for direct application and emulsifiable concentrate 20.6% (240 g active ingredient/L). The active ingredients are spinosyn A and D.

- *Preparation and use*
  - *Large surfaces, clean water (borrow pits, drains):* 20.6% spinosad emulsifiable concentrate should be diluted with water and applied at 20g active ingredient/ha, with an expected duration of efficacy of one week.
  - *Large surfaces, polluted water (cesspits):* 20.6% spinosad EC should be diluted with water and applied at 250–500g active ingredient/ha, with an expected duration of 7–10 days.

— *Container breeding:* granules or tablets should be applied by hand at 0.1–0.5mg/L. For disused wells, 20.6% spinosad EC can be diluted with water and applied at 250–500g active ingredient/ha, with an expected duration of efficacy of 11–17 days.

Insect growth regulators

Insect growth regulators should be considered for habitats which contain mosquito larvae and pupae but few other arthropods, to limit the environmental impact. They have very low toxicity to mammals, birds, fish and aquatic insects. The two groups of insect growth regulators are (i) juvenile hormone mimics such as methoprene and pyriproxyfen, which prevent the development of larvae and pupae into adults, and (ii) chitin synthesis inhibitors such as difulbenzuron and triflumeron, which kill larvae when they moult.

Methoprene

Methoprene is a juvenile hormone mimic. Methoprene briquettes can be used to pre-treat larval habitats that become inaccessible during the wet season, such as ditches, drains, catch basins, pools, tidal marshes, borrow pits and freshwater swamps. The active ingredient is relatively quickly broken down in water.

- *Toxicity*

  WHO hazard rating: unlikely to pose an acute hazard in normal use.
  Methoprene is considered by WHO to be safe for use in drinking water.

- *Formulations*

  Usually applied as briquettes containing 1.8%–8% methoprene or granules of various concentrations, which have a longer residual effectiveness:

  — *Granules and Pellets:* These are typically controlled-release formulations. The longer residual formulations can typically withstand drying and re-wetting. This makes these formulations ideal for use in habitats where flooding may be erratic or unknown.

  — Briquettes slowly release methoprene for up to four months in stagnant water and for considerably shorter periods in flowing water. Briquettes may remain effective until the next rains if a larval habitat dries up. Mud will clog briquettes and reduce their effectiveness.

- *Preparation and use*

  — *Granules/Pellets:* These formulations can be hand applied or applied with portable blowers to habitats with or without vegetation. Rates can range from 2.8–22.4 kg/ha and depend on target species/habitat and residual needs. Effectiveness lasts for 7–42 days depending on the formulation used, percentage active ingredient, rate used and the target habitat and species.

  — *Briquettes:* Applied by hand into the deepest part of a larval habitat, to sustain control during the dry season. One briquette should be placed per 10m2 in pools less than 60cm deep and with stagnant water.

Pyriproxyfen

Pyriproxyfen is a juvenile hormone mimic.

- *Toxicity*

  WHO hazard rating: unlikely to pose an acute hazard in normal use.
  Pyriproxyfen has low toxicity to non-target organisms.

- *Formulations*

  The main formulation types available are granules and emulsifiable concentrates.

- *Preparation and use*

  Large surfaces: the dosage should be 10–50g/ha.
  Container breeding: the dosage should be 0.01mg/L.

Diflubenzuron

Diflubenzuron is a chitin synthesis inhibitor used mainly to treat open larval habitats, both clear and polluted. It is effective for 1–2 weeks and up to a month in closed sites such as latrines.

- *Toxicity*

  WHO hazard rating: unlikely to pose an acute hazard in normal use.
  Diflubenzuron is considered safe for use on irrigated food crops.

- *Formulations*

  Available as a wettable powder or granules (which are for use where there is heavy vegetation or flowing water).

- *Preparation and use*

  The wettable powder is mixed with water and applied with spray equipment and granules applied by hand or portable blowers.

  Large surfaces: the dosage should be 25–100mg/ha.
  Container breeding: the dosage should be 0.02–0.25mg/l.

Novaluron

Novaluron is a benzoylurea larvicide that inhibits chitin synthesis, interfering with the moulting process.

- *Toxicity*

  Novaluron has low toxicity to birds, fish, earthworms and aquatic plants but is highly toxic to aquatic invertebrates and extremely toxic to crustaceans, however it does not persist for long.

- *Formulations*

  Available as an emulsifiable concentrate.

- *Preparation and use*

  Large surfaces: the dosage should be 10–100g/ha.
  Container breeding: the dosage should be 0.01–0.05mg/l.

4. Biological control (larvivorous fish)

Biological control is the introduction of natural enemies into larval habitats, including predatory fish, predatory invertebrates and parasites or other disease-causing organisms. While there are a large variety of available biological control agents, only larvivorous fish have been widely used. This section explains how larvivorous fish can be used in larval source management.

This manual does not specifically endorse the use of larvivorous fish. The Cochrane Review on larvivorous fish is not yet completed.

4.1 Selection of species

The following characteristics are preferable in larvivorous fish:

- Small size to allow access to all parts of the larval habitat
- Preference for mosquito larvae above other prey at the water surface
- High reproduction rate in small water bodies

- Local not exotic species
- Tolerance to salinity, pollution, temperature fluctuations and transportation

Species successfully introduced for the control of mosquito larvae (**Fig. 16**) are largely tooth carp (*Poeciliidae* and *Cyprinodontidae*), including the guppy (*Poecilia reticulata*) and the top minnow or mosquito fish (*Gambusia affinis*). *Gambusia* is more appropriate for clean water while *Poecilia* is more efficient for organically polluted water and in water of a higher temperature, such as in rice fields. Unlike *Gambusia*, *Poecilia* cannot survive temperatures below 10 °C. The annual killifishes (*Cynolebias*, *Nothobranchius* and *Aphyosemion*) have dessication-resistant eggs and are useful for larval habitats that dry out, for example borrow-pits and irrigated rice fields. The juvenile stages (but not the adults) of some species may eat mosquito larvae (5).

The geographical distribution of larvivorous fish of the tooth carp family (*Cyprinodontidae*) (5) is as follows:

- **Africa:** *Aphanius*, *Aphyosemion*, *Epiplatys*, *Nothobranchius*
- **Asia-Pacific Region:** *Aplocheilus*, *Macropodus*
- **Central and South America:** *Fundulus*, *Jordanella*, *Rivulus*, *Gambusia*, *Girardinus*, *Heterandria*, *Poecilia* (*Lebistes*), *Limia*, *Cynolebias*

Exotic species should not be introduced into natural habitats since they may displace native species or affect non-target organisms; instead, the suitability of local species should be assessed. Exotic species such as *Gambusia* may be appropriate for man-made larval habitats with no connection with the natural environment, such as water storage tanks, swimming pools, garden ponds or desert reservoirs (5).

Where there is abundant vegetation, the introduction of larger fish such as the carp (*Cyprinus carpio*), the giant gourami (*Osphronemus goramy*) or the tilapia (*Tilapia* or *Oreochromis mossambicus*) which uproot and eat plants, can help larvivorous fish to reach larvae. These larger fish can also be consumed by the local population (5).

Some fish may be reared for both mosquito control and as food, such as cichlid fish (*Oreochromis mossambicus*, *O. niloticus* and *O. Spiluris*) in Indonesia, Malaysia, Somalia and Sudan, and the common carp (*Cyprinus carpio*) and the grass carp (*Ctenopharyngodon idella*) in India and China. Larvivorous fish can also feed larger fish, which in turn may be eaten by the local population (5).

4.2 Rearing fish

Where larvivorous fish are found naturally in particular locations, these can be used as a source. To ensure a large supply of fish they should be reared in large quantities in special breeding ponds. Ponds used to rear fish for food can be simultaneously used to rear larvivorous fish. Ponds dug out of the earth or large cement tanks can be used to rear fish. Plenty of space and aquatic vegetation are essential to protect younger fish from older fish. Feeding with organic waste or animal manure can increase production, but the growth of algae (which leads to de-oxygenation) should be avoided, e.g. by using a herbicide (5). Care should be taken not to create mosquito habitats while digging the ponds.

4.3 Transportation and distribution

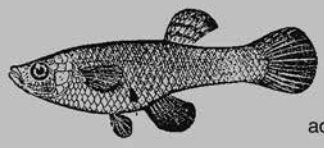
Fish should be transported in containers with a volume of up to 40 litres (e.g. plastic buckets, jerry cans) closed and two thirds full, or in strong plastic bags half full of water from the rearing pond. Numbers should be kept low in each container, e.g. 50 *Gambusia* in 8L water. Temperature should be kept steady (e.g. by wrapping containers in wet cloth, or placing containers in wooden boxes or polystyrene boxes) and oxygen kept in adequate supply. Water from the final destination should be added to these containers before releasing fish to allow them to gently acclimatise. Six *Gambusia* would be sufficient for a pool of 5–10 m^2 with few aquatic plants (5).

Figure 16. Larvivorous fish species, adapted from (*5*)

Effective larvivorous fish species

The mosquito fish or top minnow, Gambusia affinis

This species is the most widely used against mosquito larvae. Together with the guppy it belongs to the live-bearing tooth carp family, Poeciliidae. Their mouths are adapted to feeding from the surface. It originates from Central America but, because of its success in controlling mosquitos, has been introduced into many parts of the world. These fish can withstand large fluctuations in temperature as well as pollution of the water, but they are most productive in relatively clean water of moderate temperature.



actual size

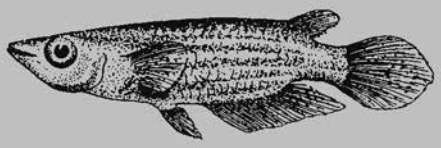
The guppy, Poecilia reticulata

Similar to the mosquito fish, this is a live-bearing tooth carp that is adapted to taking food from the surface. It originates from South America and has become very popular as an aquarium fish. It has been introduced for mosquito control in many countries, especially in South America and Asia. The species prefers higher temperatures than the mosquito fish and can withstand highly polluted water. It has therefore been most successful against *Culex* mosquitos which breed in organically polluted water.

actual size

The panchax, Aplocheilus panchax

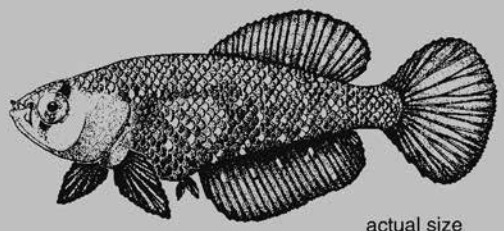
This egg-laying tooth carp is found in the Indian subcontinent, Indonesia, Malaysia and Sri Lanka, where it commonly occurs in paddy fields and ditches and is important in the control of mosquitos. The fish can withstand pollution and water temperatures between 20 °C and 45 °C.



actual size

The Argentine pearlfish, Cynolebias bellotii

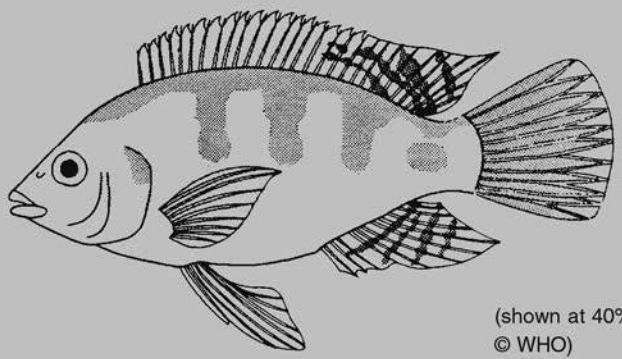
This is one of the annual fishes that occur in South America and Africa, known as instant fish. They cannot reproduce in permanent water bodies and occur only in habitats where the water disappears every 2–3 months or at least once a year. The eggs, which survive the dry period buried in the soil, may be concentrated, transported and dispersed in slightly damp material. They hatch within a few hours after flooding. Although not extensively evaluated, these fish may be useful in borrow-pits and temporary dry pools as well as in rice fields and irrigated pastures where other fish cannot survive.



actual size

The Mozambique mouthbrooder, Oreochromis (Tilapia) mossambicus

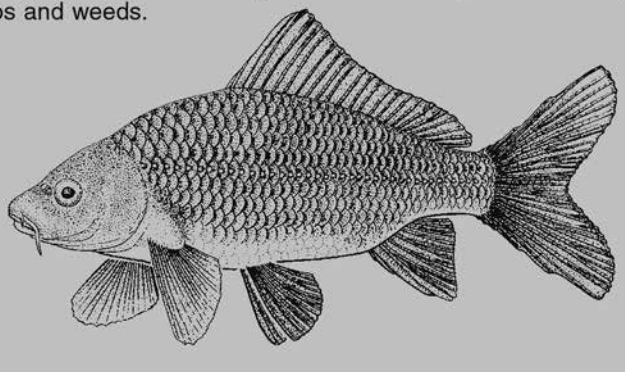
This cichlid fish occurs in East Africa. It has been reared successfully in irrigated rice fields where it was used both to control mosquitos and as a source of food. With an optimal temperature of 22 °C it reproduces very rapidly. The species can live and reproduce in fresh and brackish water.



(shown at 40%, actual size is 20 cm;
© WHO)

The carp, Cyprinus carpio

This edible fish can be reared in irrigated rice fields, ditches and ponds; it is hardy and prefers rich, shallow waters with muddy bottoms and good aquatic weed growth. The species multiplies when the water temperature is over 18 °C. The fingerlings feed on mosquito larvae, the adults on aquatic vegetation, weeds and algae but not on rice plants. The carp can be used to control both mosquitos and weeds.



(shown at 25%, actual size is 32 cm).

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Annexes

Annex 1

BIOLOGICAL INFORMATION

This annex provides important biological information on mosquito bionomics and malaria transmission, an understanding of which is essential to assessing whether LSM is appropriate, and for running a successful LSM programme.

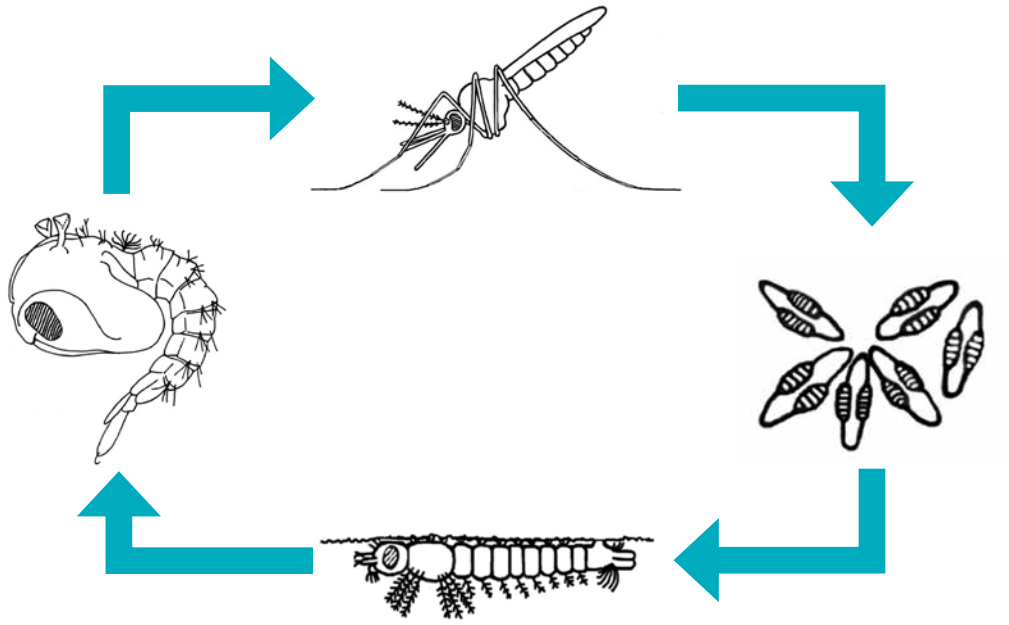
1. Mosquito bionomics

Malaria is transmitted by females of the *Anopheles* genus (anophelines). Other genera of medical importance are *Aedes* in the subgenus *Stegomyia* (which transmit Yellow Fever, Dengue Fever, filarial worms), *Culex* (filarial worms, Japanese encephalitis) and *Mansonia* (filarial worms). Mosquitoes of these three genera belong to the culicine subfamily. This annex outlines the life cycle of anopheline larvae and describes how anopheline mosquitoes can be morphologically distinguished from culicines.

Life cycle and morphological features of target larvae

The life cycle of the mosquito has four stages: egg, larva, pupa and adult (**Fig. 1**). The first three stages (egg, larva and pupa) are aquatic and generally last 5–14 days, depending on the ambient temperature and species. Adults can survive for up to one month but generally live for 1–2 weeks.

Figure 1. Anopheline mosquito life cycle. Source: C. Whitehorn.



Eggs

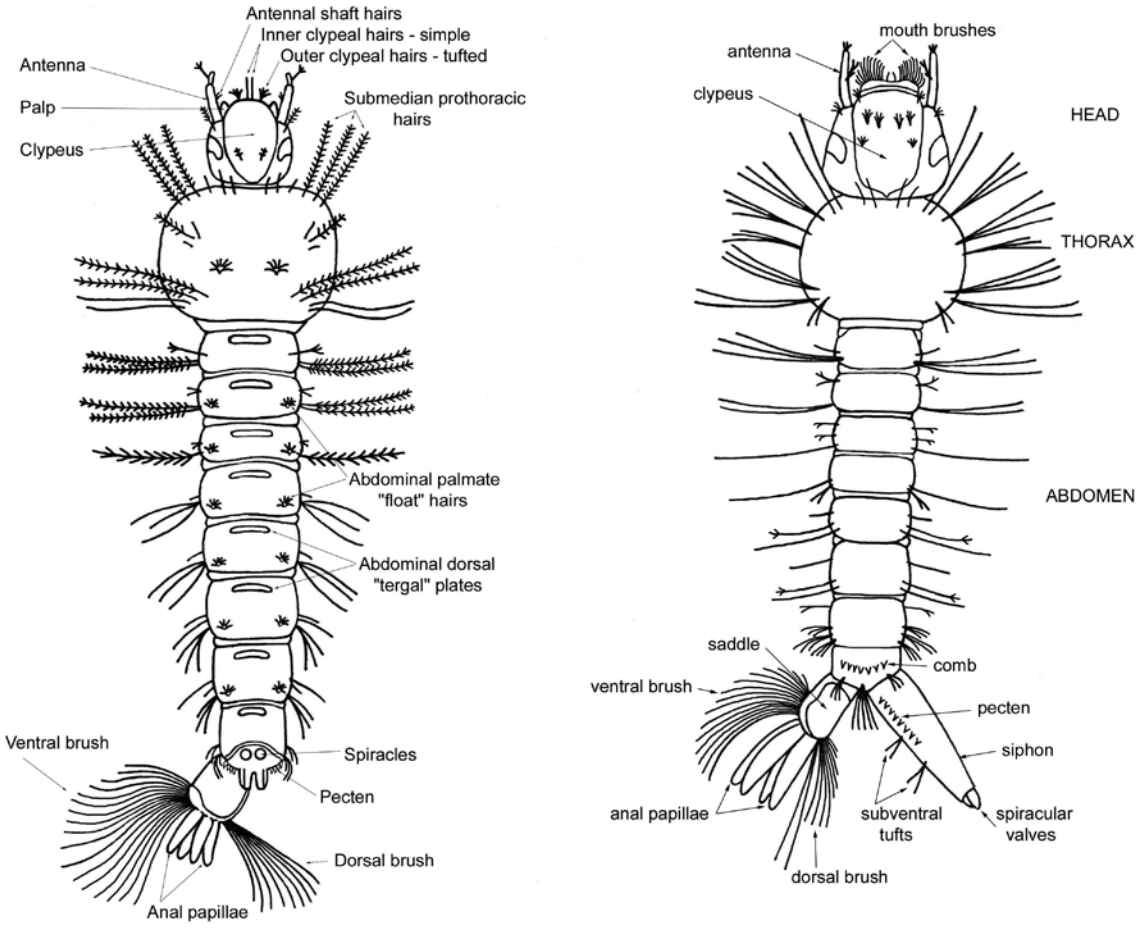
The adult female lays on average 50–200 eggs per oviposition, singly onto water. Eggs are characterized by their lateral floats (**Fig. 2**) and attach by surface tension to the water surface or objects in the water. If eggs dry out the mosquito will not develop. Eggs typically hatch within 2–3 days. However, hatching may take 2–3 weeks in colder regions.

Figure 2. Anopheles eggs. Note these are laid singly. Source: C. Whitehorn.

Larvae

Larvae emerge using an egg tooth located on the head. They then develop through four stages or instars which are increasingly large, before metamorphosing into pupae after 5–10 days. Before moving to the next stage, larvae moult and shed their exoskeleton (skin) to allow growth. First instar larvae are very small and this means they are often overlooked. Larvae have a head with mouth brushes for feeding, a thorax and a segmented abdomen. Anopheline larvae do not have a respiratory siphon, which means that unlike culicine larvae which hang perpendicular to the water surface (**Fig. 5**) they must position themselves parallel to the water surface to breathe via spiracles on their abdomen (**Fig. 3**). Larvae swim backwards near the water surface until contact is made with a solid object, which they then lie against. They dive below the surface when disturbed. They feed on microorganisms in the surface microlayer such as algae and bacteria.

Figure 3. Anopheles larva (left). Note absence of siphon and subventral tufts that characterize Culicine larvae (right). Source: C. Whitehorn.



Pupae

When viewed from the side, the pupa is shaped like a comma (**Fig. 5**). Pupae must visit the water surface to breathe via a pair of respiratory trumpets on the cephalothorax (merged head and thorax). Pupae are very active and dive below the water surface at the slightest disturbance of the water. The pupal stage lasts from a few hours to a few days, then the surface of the cephalothorax splits and the adult mosquito emerges.

Adults

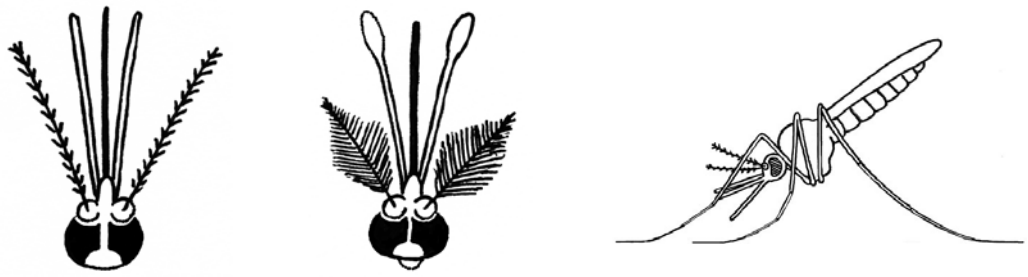
Adult *Anopheles* mosquitoes have three body sections: the head, thorax and abdomen. Attached to the thorax are three pairs of legs and one pair of wings (**Fig. 5**).

Anophelines can be distinguished from other mosquitoes by:

- Palps, which are the same length as the proboscis (**Fig. 5**).
- Wings, which have discrete blocks of black and white scales (**Fig. 5**).
- Resting position, with their abdomens (rear ends) pointing upwards rather than held parallel to the ground (**Fig. 4**).

Female anophelines can be distinguished from males by their antennae, which are less hairy than male antennae (**Fig.4**).

Figure 4. Anopheles adult female head (left), Anopheles adult male head (centre), Anopheles adult (right). Note the typical adult resting position, with abdomen pointing upwards (right).
Source: C. Whitehorn.



The time it takes for adult mosquitoes to mate after emerging from pupae varies from a few hours to several days. Males live for approximately one week and feed on nectar and other sources of sugar. Although females feed on sugar, they require a blood meal for their eggs to mature. After acquiring a full blood meal, females usually rest for 2–3 days in tropical conditions while their eggs mature then eggs are laid and the female resumes host seeking. The cycle of feeding, resting and ovipositing is then repeated until the female dies. The longevity of the female depends on temperature, humidity and success in obtaining blood meals.

Larval habitats

Anopheline larvae are found in a wide range of habitats worldwide (e.g. rice fields, ditches, puddles, the edges of streams and rivers, fresh and brackish marshes and mangrove swamps) but generally tend to avoid highly polluted water. **Table 1** summarizes the habitats and habitat characteristics of the major malaria vectors of Africa (*1*).

The abundance of adult mosquitoes depends on the number, type and size of potential larval habitats, their distance from blood meal sources, the density of larvae in the larval habitats, temperature, rainfall and soil type. Larval habitats may be stable or dynamic, appearing briefly after rainfall.

Figure 5. Morphological differences between Anopheline and Culicine mosquitoes. Source: C. Whitehorn.

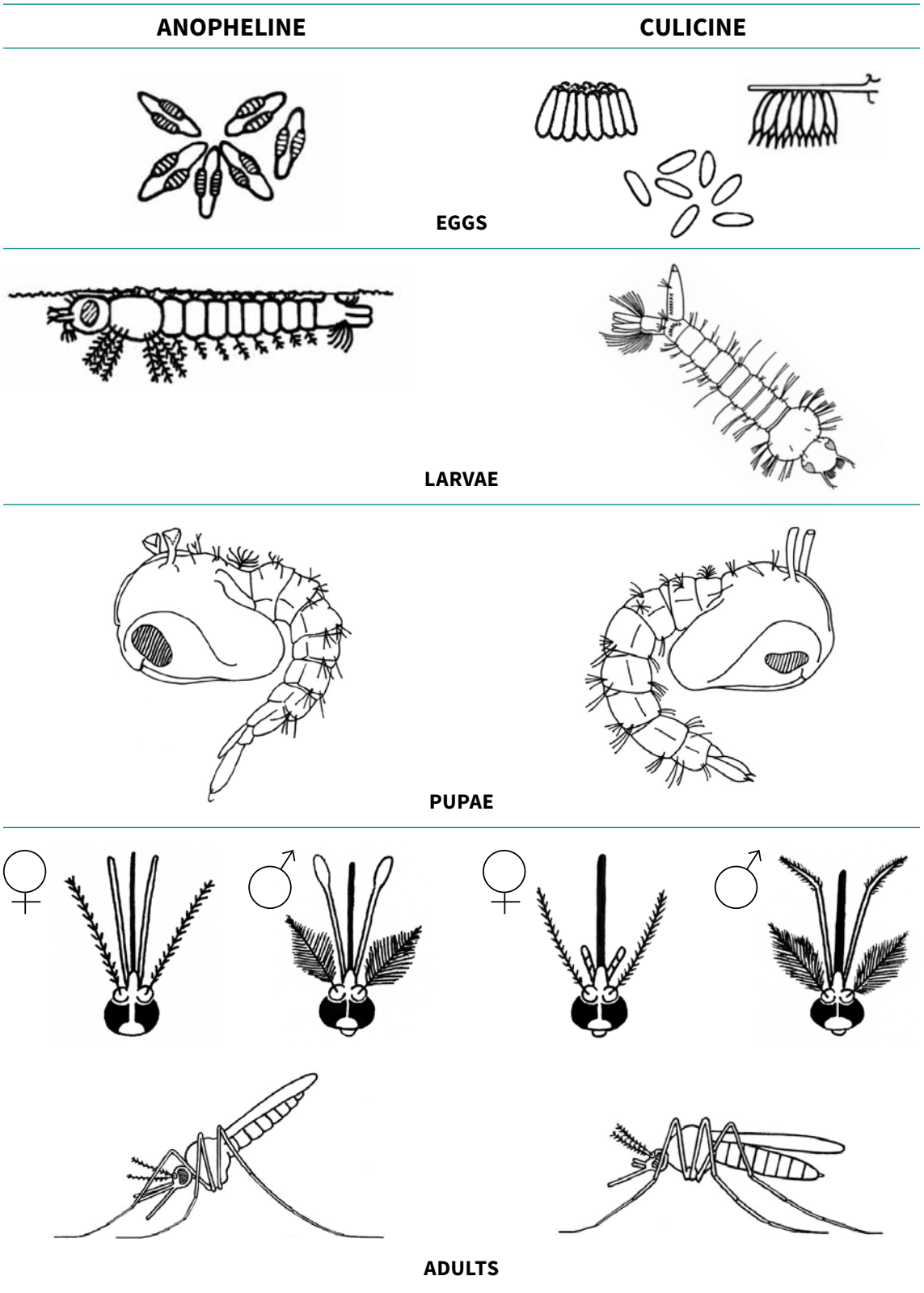


Table 1. Typical larval habitats and larval habitat characteristics of major malaria vectors of Africa (*1*)

SPECIES	TYPICAL LARVAL HABITATS				LARVAL SITE CHARACTERISTICS
	LARGE NATURAL WATER BODIES	LARGE MAN-MADE WATER BODIES	SMALL NATURAL WATER BODIES	SMALL MAN-MADE WATER BODIES	
An. funestus	Edges of dykes, marshes, slow flowing rivers	Rice fields, fish ponds, irrigation channels	Small streams, seepage springs, pools, wells	Overflow water, irrigation ditches	Sunlit, shaded fresh
An. arabiensis, An. gambiae s.s.	Edges of lakes, marshes	Borrow pits, rice fields, fish ponds, irrigation channels	Small streams, seepage springs, pools, wells	Overflow water, irrigation ditches, borrow pits, wheel ruts, hoof prints, rice field puddles	Sunlit, fresh & polluted
An. melas	Lagoons	–	Pools	–	Sunlit/shaded, brackish
An. merus	Lagoons	–	Pools	–	Sunlit/shaded, brackish
An. moucheti	Lakes, slow flowing rivers	Rice fields, fish ponds	small streams, pools	–	Sunlit, fresh
An. nili s.l.	Slow flowing rivers	Rice fields, irrigation channels	Small streams	irrigation ditches	Shaded, fresh

Adult mosquito habitats and behaviour

Mosquitoes typically disperse over several kilometres from their breeding sites. Adult male mosquitoes are weaker fliers than females, therefore a high density of adult male mosquitoes indicates that their breeding place is relatively close.

Mosquitoes have very different biting and resting habits that need to be understood when making decisions about malaria vector control. Mosquitoes that feed and rest indoors are known as endophagic and those which feed and rest outdoors are exophagic. Feeding patterns vary with species, but in general biting tends to occur between dusk and dawn (and especially during the early hours of the morning, when the air is most humid) in species that are associated with open terrain or sunlit habitat. This is true of *An. gambiae* s.l. and *An. funestus* whose peak biting occurs an hour before dawn (*2*).

Endophagic mosquitoes may rest indoors after feeding for a few hours, before either returning outdoors to rest or remaining indoors to digest their blood-meal and produce eggs. Once eggs have developed the gravid mosquitoes leave their resting sites and look for a suitable larval habitat (*3*).

House design can influence mosquito house entry, e.g. in the Gambia where *An. gambiae* enters houses through open eaves, features such as closed eaves; concrete walls or house screening make entry more difficult (*4,5*). Even where species are predominantly endophilic, there will still be an outdoor resting population composed of males, newly emerged females, gravid females and females which have just oviposited. When a species is predominantly exophilic, the proportion of the outdoor population that is recently blood-fed is higher. Outdoor resting places are typically sheltered, shaded or humid and preferences vary between species, as does the preferred type of vegetation for resting on.

Anopheline species differ in the extent to which they prefer to feed on humans and animals. Species with a greater preference for humans are known as anthropophilic (and are more efficient vectors) and those with a lesser preference for humans are known as zoophilic.

Table 2. Biological information related to the vectorial efficiency of the major malaria vectors of Africa (*1*)

SPECIES	FEEDING PREFERENCES		PERDOMINANT BITING HABIT		PRE-FEEDING RESTING HABIT		POST-FEEDING RESTING HABIT	
	ANTHROPO-PHILIC	ZOO-PHILIC	EXO-PHAGIC	ENDO-PHAGIC	EXO-PHILIC	ENDO-PHILIC	EXO-PHILIC	ENDO-PHILIC
AFRICA								
An. arabiensis	Yes	Yes	Yes			Yes	Yes	Yes
An. funestus	Yes			Yes	Yes	Yes		Yes
An. gambiae s.s.	Yes			Yes		Yes	Yes	Yes
An. melas	Yes	Yes	Yes		Yes	Yes	Yes	Yes
An. merus	Yes	Yes	Yes		Yes	Yes	Yes	Yes
An. moucheti	Yes			Yes	Yes	Yes	Yes	Yes
An. nili s.l.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

2. Malaria transmission

Malaria is transmitted when an adult female anopheline mosquito takes a blood meal from an infected human. The parasite gametocytes are ingested by the mosquito and reproduce in the gut, forming sporozoites that migrate to the salivary glands. When the mosquito takes a second blood meal, the sporozoites are injected into the human. These enter the liver, undergo further development and subsequently enter the bloodstream and infect red blood cells; infected red cells later burst, releasing parasites which infect other blood cells and produce the symptoms of malaria.

The level of malaria transmission is determined by the number of infective bites received per person per year, a rate described as the entomological inoculation rate (EIR). EIR is the product of the human biting rate and the proportion of mosquitoes with sporozoites in their blood:

EIR = sporozoite rate x human biting rate

LSM reduces the overall density of adult vectors, reducing the human biting rate. It therefore can have an additive benefit in reducing malaria transmission when integrated with LLINs and/or IRS in certain settings (*6–8*).

The theoretical measure of malaria transmission intensity is R_0, the basic reproductive number ('the expected number of hosts who would be infected after one generation of the parasite by a single infectious person who had been introduced into an otherwise naïve population'(*9*)), which was first formalized in the Ross-Macdonald model (*10*). This model demonstrated that the intensity of malaria transmission is determined by the density of adult female anophelines, but also and more importantly by the human biting rate and the daily survival rate of the adult mosquito. Thus, targeting the adult vector has a greater effect on malaria transmission than targeting larval stages. Consequently, malaria vector control in the last half century has largely focused on IRS, and more recently LLINs, since they affect daily adult survival rates. However in practice, interventions that target the adult vector, such as LLINs and IRS, may reach a maximum practical effect, due to insecticide resistance, avoidance behaviour by mosquitoes, outdoor biting, or low LLIN use by people. Where larval habitats are obvious, discrete and readily accessible, they may be appropriate targets for controlling vectors. Therefore LSM may complement LLINs and IRS in appropriate settings through its direct effect on the density of adult anophelines. **Table 2** demonstrates how biting and resting behaviour differs between major vector species, e.g. *An. arabiensis* frequently feeds and rests outdoors therefore is difficult to target with LLINs and IRS.

Because LLINs and IRS have a varying impact in different locations, due to variation in the baseline level of transmission and heterogeneous biting, complementary interventions such as LSM may play a supplementary role in some settings. R_0 varies considerably and has been estimated to range from 1 to 3000 in 121 African populations. Where transmission is 'stable' the value of R0 is

high, exceeding the value needed to sustain transmission, and acquired immunity to severe disease limits the number of new cases observed (*11,12*). In such areas, control interventions must be highly efficacious to reduce malaria morbidity. Heterogeneous biting also reduces the 'efficiency' of transmission at high transmission intensity, which similarly means that a reduction in transmission will not necessarily produce a reduction in malaria morbidity (*13,14*). Heterogeneous biting is the variation in the distribution of bites (as a working approximation, 20% of the population receives 80% of bites (*14,15*)), which arises due to factors such as uneven use of protective clothing or LLINs or differences in attraction or exposure to mosquitoes. Therefore, in areas of high endemicity, LLINs and IRS may need to be supplemented with other interventions to have the desired impact on malaria morbidity.

Annex 2

ADULT MOSQUITO SURVEYS

Overviews of adult mosquito survey methodologies include:

- WHO (1992) *Entomological field techniques for malaria control Part I. Learner's guide*, WHO, Geneva.
- Silver JB, (2008) *Mosquito ecology: field sampling methods*, Third Edition, Chapman & Hall, London.
- Service MW, (1996) *Medical Entomology for students*, Chapman & Hall, London: 289P.

Adult surveys should be conducted over a representative period during the transmission season in order to understand the population dynamics. Use should be made of any data already available from the operational setting from earlier studies.

A well-designed survey for adult *Anopheles* will include traps as well as methods of capturing the resting adults. The resting behaviour of *Anopheles* after blood feeding provides a very good opportunity for effective survey and identification of the key problem vectors, because it will enable the collector to find blood-fed adults and to determine which hosts have been fed upon. Collection of adults during host seeking is also useful, but requires considerable evening work and involves a risk of receiving bites.

Light Traps: The CDC light trap (**Fig. 1**) and traps of similar design have proven to be effective tools for monitoring Anopheles populations and generally attract different numbers of mosquitoes than human landing catch in most African settings (*16,17,18*). These devices are hung in a room with a person sleeping under an untreated net and the light attracts mosquitoes. This simulates the number of bites received per person per night. Mosquitoes are sucked into a net bag by an electric fan. Light traps are useful for obtaining an overall picture of the population and to monitor population changes over time. Since attraction to light is primarily a host-seeking behaviour, the traps tend to catch many mosquitoes prior to taking a blood meal.

Figure 1. CDC Light Trap.
Source: S. Lindsay

Pyrethrum Spray Catch (PSC): This method takes advantage of the tendency of malaria vectors to rest inside houses. Plastic or cloth sheeting is laid down in a house, and pyrethrum insecticide is then applied as an aerosol (**Fig. 2**). The mosquitoes killed and knocked down by the spray are collected. Since the mosquito will rest on the ceilings and walls of houses after taking a blood meal, this method tends to catch more blood-fed individuals.

Human landing catch (HLC): During a human landing catch, an aspirator is used to collect mosquitoes approaching or landing on humans for a blood meal (**Fig. 3**). The method provides a very

Figure 2. Pyrethrum spray catch.
Source: S. Lindsay (left), Gates Malaria Partnership Vector Biology Course (right).

clear picture of which species are blood feeding on humans in the specific location. These surveys require staff to work in the evening and are time consuming. In some countries, particularly where there are circulating arbo-viruses such as Rift Valley Virus, National Research Ethics committees have discouraged the use of Human Landing collections. In other countries, collectors are provided with malaria prophylaxis and close follow-up. Programmes should check with their national research ethics committees if and how Human Landing Collections can be implemented.

Figure 3. Human landing catch.
Source: S. Lindsay

Different methods of adult mosquito collection only work under specific conditions. For example, pyrethrum spray catch is less fruitful in areas with low vector densities and high volumes of insecticide-treated material inside houses. Therefore, if a particular method is not collecting many specimens, alternative tools should be considered.

Identification of specimens

Keys to anopheline mosquitoes of Africa are available, however specific identification is complex and will probably require molecular methods to distinguish between closely related and morphologically similar groups. This is important information to allow the main vector to be identified. Sporozoite infection rate (the percentage of mosquitoes with sporozoites in their salivary glands) should also be determined using dissection or by enzyme-linked immunosorbent assay (ELISA).

Annex 3

LARVAL MOSQUITO SURVEYS

With the aid of existing historical information about adult populations and current information from on-going adult surveys, it is possible to design and carry out a programme of larval surveillance and larval source identification.

The information from larval surveys is of general use to a malaria control programme, not only for LSM. For example, it can help forecast the need for adult mosquito control, assess the effectiveness of adult control measures, help interpret adult mosquito surveillance data and detect insecticide resistance.

A thorough survey will over time provide a clear picture of where, when and under what conditions larval development occurs. It will be therefore necessary to track rainfall, and in some situations, such as southern Africa during winter, temperature data during larval surveys. Other important data, depending on the situation, will include irrigation practices, river levels and tides that may impact larval sources. Human activities such as construction, brick manufacturing and well digging will also play a role. Soil type will determine how long areas remain flooded after rainfall or if surface water will develop from elevated water tables. As each local situation will be unique, the possibility of other sources should always be considered. Importantly, ***all accessible water bodies need to be searched for mosquitoes***, to ensure that all larval habitats are identified and can be treated.

To identify larval habitats, it is essential to check all possible sites, even those that are difficult to reach. Potential larval habitats include:

- Small rain pools, hoofprints, drains and ditches, where the entire surface of the water should be examined;
- Brackish water (where fresh and salt water mix);
- Streams, which should be searched at the edges where there is vegetation and where water moves more slowly;
- Ponds, lakes, swamps and marshes where larvae usually occur in vegetation around the edges, but can sometimes be found far from the shore amongst floating vegetation;
- Special sites, such as wells, abandoned swimming pools and water containers made of cement, where the entire surface of the water should be examined;
- Drains in urban and peri-urban areas and alongside roads.

Surveys of larval populations need to be conducted in a systematic manner so that all larval sources may be logged and categorized. Larval sources within a prescribed radius of the protected area also need to be characterized in terms of ecological triggers that support larval development. These data need to be organized collectively in an accessible format such as a computer database. If available, a geographic information system (GIS) should be used for this purpose since this

allows the visualization of the data and integration with databases from other entomological and epidemiological surveys.

Modern GIS techniques are very useful for data retrieval and information tracking, but are not essential to programme development. However, detailed maps and a clearly delineated system of numbering and logging larval data are very important. These data will be essential to making decisions about the feasibility of LSM in a particular setting and in planning and carrying out operations.

Knowledge of the ecology of the local vector species will aid in identifying larval sources. A brief description of selected larval habitats of malaria vectors is provided in **Annex 1**. This summary of information implies that general trends exist, but local conditions may be different and there is no substitute for a thorough local survey.

Larval sampling methods

Mosquito larvae can be sampled using various methods, but the most practical is the use of dippers (*19*). Mosquito dippers of various shapes and sizes may be employed. A 'standard dipper' consists of a white plastic, metal or porcelain cup attached to the end of a stick (**Figs. 1, 2**). The cup usually has a capacity of 350 to 400 ml and has a drain spout for transferring the sample to collection vessels for return to the laboratory.

Staff employed in a larval survey using dippers should be trained in their proper use. An effort should be made to standardize their methods to keep data consistent. A standard number of dips should be taken per unit area. Often it is best to work in transects, taking one dip for a given number of steps.

Figure 1. Larval surveys. Source: S. Lindsay

Potential larval habitats should be approached slowly and carefully and facing the sun, to avoid heavy footsteps or casting a shadow over or disturbing the water, which may cause larvae to dive to the bottom. Larvae are generally found at the surface, close to vegetation or floating debris and at the edges of larger and deeper water bodies. Dipping should be conducted close to floating debris and vegetation, on the windward site of the habitat where larvae and pupae will be concentrated, and not during rainfall. Water bodies often constitute a variety of microhabitats (e.g. open water, under floating vegetation) containing dif-

Figure 2. Dippers for larval surveys. Source: S. Lindsay (left); University of Florida (right).

ferent mosquito species. These should all be sampled to obtain an accurate picture of the species composition of the area (*20*).

Anopheles larvae are generally best collected using the 'shallow skim' approach. The dipper is tilted by 45 degrees and its leading edge submerged two centimetres below the water surface. The dipper is moved quickly but gently in a straight line until the dipper is full but not overflowing (*20*).

Nets are also useful for collecting *Anopheles* spp. larvae and have been shown to be more efficient (**Fig. 3**), especially for collecting pupae (*21*). However, for durability in routine operational use, dippers are generally preferred.

Personnel will need basic training in recognition of mosquito genera in the larval stage. Most important is the ability to distinguish between *Anopheles*, *Culex* and *Aedes* mosquitoes. More information on differences between species is given in **Annex 1**.

Figure 3. Using a net to sample larvae.
Source: Vincent Robert, *Malaria Journal* 2002

Data on both habitat occupancy and larval density should be collected:

- **Habitat occupancy:** The presence or absence of larvae in a breeding site is determined by visual observation. If a habitat is positive (i.e. larvae are present), the next step is to determine larval density.
- **Larval density:** A number of dips should be taken from the habitat and the number of larvae collected should be counted. All the larvae present should be counted. The number of dips to be taken will depend on the size of the breeding site. A footprint or hoofprint is considered as one dip. For larger breeding sites such as borrow pits, it is recommended that one dip is taken per square metre of surface, up to a maximum of 30 dips.

Other information collected should include: GPS coordinates of larval source, a site designator (number), habitat type, size of habitat, depth and vegetation type and density, numbers of each larval instar collected per dip. A sample form for data recording is shown in **Fig. 4**.

LARVAL INSPECTION FORM																
INSPECTOR NAME			HABITAT DETAILS				SAMPLING RESULTS								% VEGETATION COVER & TYPE
DATE							LARVAL STAGES PRESENT (YES/NO)					DIP COUNT (add # pupae)			
ZONE															
TIME	SOURCE NUMBER	LOCATION DESCRIPTION	LAT	LON	HABITAT TYPE	SQUARE METERS	L1	L2	L3	L4	PUPAE	# DIPS	# An	# Cx	
							An	Cx	An	Cx	An	Cx	An	Cx	

Figure 4. Example of larval inspection form (*22*)

Annex 4

LARVAL SURVEILLANCE FORM AND SUPERVISOR SPOT CHECK FORMS

The Urban Malaria Control Program, Dar es Salaam

Ward level mosquito larval habitat survey - Open habitats

Serial number of this form ______________________

Serial number on the map form ______________________

Ten cell unit identifier ______________________

Municipality:____________________ Ward:______________________ Mtaa/area:______________________ 10-cell unit:____________________ 10-cell leader:______________________

Habitat codes:

1: Puddles&tire tracks
2: Swampy areas
3: Mangrove Swamp / Saltwater marsh
4: Drain/Ditch
5: Construction pits/foundations/man-made holes
6: Water storage container
7: Rice paddy
8: Matuta
9: Other agriculture
10: Stream/river bed
11: Pond
12: Other (describe below)

Plot ID	Habitat ID	Habitat type	Same habitat type from last visit? 1=Yes 2=No 3=First visit	Previous habitat type	Habitat description	House number	Wet?		Habitat perimeter			Plants				Water depth		Larval stage						Pupae		Comments
																		Anoph.			Culex					
							dry	Contains water	< 10 m	10-100 m	> 100 m	None	Short vegetation	Tall vegetation	Floating plants	< 0.5 m	> 0.5 m	Absent	Early	Late	Absent	Early	Late	Absent	Present	

CORP's signature:________________________ _______ Date:_______/_______/_________ Supervisor's signature:________________________ Date of check :_______/_______/_________

Ward level mosquito larval habitat survey - Closed habitats

Serial number of this form _____

Serial number on the map form _____

Ten cell unit identifier _____

Municipality:_____ Ward:_____ Mtaa/area:_____ 10-cell unit:_____ 10-cell leader:_____

Habitat codes: 1 - Pit Latrine 2 - Soakage Pit 3 - Septic Tank 4 - Other_____

Plot ID	House number	Habitat ID	Habitat type	Habitat description	Wet?		Condition of the latrine			Habitat perimeter			Water depth		Larval stage			Pupae		Comments
					dry	contains water	Good	Bad	Full	< 10 m	10-100 m	> 100 m	< 0.5 m	> 0.5 m	None	Early	Late	Present	Absent	

CORP's signature:_____ _____ Date:_____/_____/_____ Supervisor's signature:_____Date of check :_____/_____/_____

Municipal mosquito larval habitat spot check - Open habitats

Serial number of this form ______________________

Serial number on the map form ______________________

Ten cell unit identifier ______________________

Municipality:____________________ Ward:______________________ Mtaa/area:______________________ 10-cell unit:____________________ 10-cell leader:______________________
GPS(UTM/WGS84): Northing________________________ Easting______________________________ Date of CORP's data sheet:________/________/__________
Serial number on the map form__________________________

	Yes	No
Is there a map?		
Is the map accurate?		
Does map match city copy?		

Habitat codes:

1: Puddles/tire tracks
2: Swampy areas
3: Mangrove Swamp
4: Drain/Ditch
5: Construction pits/foundations/man-made holes
6: Water storage & any other man-made container
7: Rice paddy
8: Matuta
9: Other agriculture
10: Stream/river bed
11: Pond
12: Others (describe below)

Plot ID	House No.	Habitat ID	Habitat type	Is habitat type correct?	Correct habitat type	Habitat found by the CORPs? 1=Yes 2=No	Habitat Description	Wet?		Habitat perimeter			Plants				Water depth		Larval stage Anoph.			Larval stage Culex			Pupae		Comments
								dry	contains water	< 10 m	10-100 m	> 100 m	None	Short vegetation	Tall vegetation	Floating plants	< 0.5 m	> 0.5 m	Absent	Early	Late	Absent	Early	Late	Absent	Present	

Inspector's signature:____ Date of check :______/______/_______

Municipal Coordinator's signature:________________________________ Date of check :______/______/_______

Municipal mosquito larval habitat spot check - Closed habitats

Serial number of this form ______________________

Serial number on the map form ______________________

Ten cell unit identifier ______________________

Municipality:____________ Ward:_____________ Mtaa/area:_____________ 10-cell unit:____________ 10-cell leader:_____________
GPS(UTM/WGS84): Northing _____________ Easting_________________

	Yes	No
Is there a map?		
Is the map accurate?		
Does map match city copy?		

Serial number on the map form________________

Habitat codes: 1 - Pit Latrine 2 - Soakage Pit 3 - Septic Tank 4 - Other____________

Plot ID	House No.	Habitat ID	Is habitat type correct?	Correct habitat type	Habitat found by the CORPs? 1=Yes 2=No	Habitat Description	Wet?		Condition of the latrine			Habitat perimeter			Water depth		Culicine stage			Pupae		Comments
							dry	contains water	Good	Bad	Full	< 10 m	10-100 m	> 10 m	< 0.5 m	> 0.5 m	None	Early	Late	Absent	Present	

Inspector's signature:______________________ Date of check :_____/_____/______ Municipal Coordinator's signature:____________________ Date of check :_____/_____/______

Annex 5

STANDARD OPERATING PROCEDURES FOR LARVICIDING

This document has been produced and made available by the Dar es Salaam Urban Malaria Control Programme. Contact: Urban Malaria Control Programme, City Medical Office of Health, City Council, P.O. Box 63320, Dar es Salaam, Tanzania, Phone: +255 22 212 1649

Larval Control Interventions in Dar es Salaam GUIDELINES

Background Information and Operational Procedures, March 2006

First Intervention Phase March 2006 to March 2007: Intervention Ward Selection

Following preliminary data analyses and field visits three wards have been selected as intervention sites (one from each municipality) for 2006 while the other 12 wards are to be left as untreated controls.

Of these untreated control wards, where no insecticides will be applied this year, three (one from each municipality) have been selected for comparison with the intervention wards for final analyses. The selection of the sites was based on the following observations:

During the baseline data collection period the study wards were seen to differ greatly in the number of potential malaria vector habitats available, in the proportion of available habitats colonized by *Anopheles* larvae, in the density and seasonality of adults found in houses, and in malaria prevalence. The research team based the decision on which wards should receive larviciding and which wards will be compared with the intervention wards primarily on the proven ability of the ward supervisors and ward-based CORPs to implement the required task. Specifically, their ability to collect, understand, use and submit high quality data during the baseline data collection period was the primary criterion for choosing these high priority wards.

Specific Objectives of the Mosquito Larval Control Pilot Studies in 2006

- To identify and characterize all potential aquatic habitats of culicine and vector anophelines in the study wards and to study their seasonal distribution;
- To study seasonal larval population dynamics of Culex and vector anophelines;
- To establish the level of biting intensity by anopheline and culicine mosquitoes and determine human malaria exposure, measured as the entomological inoculation rate (EIR) during the dry and rainy seasons;
- To determine the prevalence of malaria infections in the population;
- To implement the microbial larval control intervention in three study communities (wards);
- To ensure community consent and cooperation.

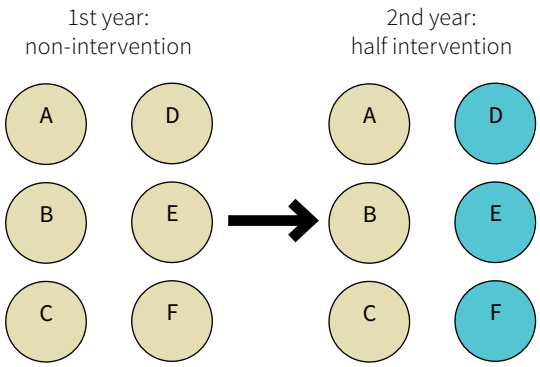
Study Hypothesis

Larval mosquito control in urban Dar es Salaam where malaria transmission is relatively low and focal will decrease densities of adult mosquitoes to such an extent that malaria transmission will also decline and reduce the level of malaria infection prevalence in local communities/wards where larviciding takes place.

Timeline

- Collection of baseline data from March 2005 to February 2006:
  - Availability of aquatic habitats (weekly);
  - Colonization of habitats with Anopheles mosquitoes (weekly);
  - Adult mosquito densities in houses (weekly);
  - Malaria prevalence and incidence in population (twice per year in each ward).
- Training on application of microbial larvicides in February 2006.
- Implementation of weekly monitoring and larviciding in intervention sites from March 2006 to March 2007:
  - Monitoring and Evaluation of intervention from March 2006 to March 2007 will use the same surveillance system described above for the baseline period.

Pilot study design



Site Selections

Non-Intervention Sites
- Keko
- Vingunguti
- Mwananyamala

Intervention Sites
- Kurasini
- Buguruni
- Mikocheni

Bacillus formulations – Background

- Discovery of the mosquitocidal Bacteria strains of *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) during the mid-1970s.
- Advantages of microbial larvicides:
  - highly effective (need very little to kill mosquito larvae);
  - selective in action (kill only mosquito and blackfly larvae in recommended dosage);
  - environmentally safe to non-target organisms (other organisms living in water such as those that feed on mosquito larvae will not be killed);
  - safe for human handling and consumption: Microbials are natural mosquito diseases that can in no way harm humans. In fact **WHO recommends them for drinking water**;
  - easy and safe to handle.
- Resistance: *Bs* can introduce resistance but this can be reversed by rotating with an alternative insecticide. Resistance to *Bti* has **never** been observed in over 30 years of use around the world.

Bacillus formulations – Mode of action

- Bacillus is a bacteria that forms spores when conditions become adverse;
- During formation of spores a special protein is produced;
- This protein is toxic to mosquito larva but only when eaten by them;
- The mosquito-killing protein is activated by digestive enzymes and alkaline pH in midgut of the mosquito larvae;
- These special proteins then attack the midgut causing the formation of pores (small holes) and destruction of the cells that line the midgut;
- Midgut pH drops to neutral;
- Larvae can no longer digest food and subsequently die;
- Only mosquito and blacklfy larvae provide conditions in the gut to activate the mosquito-killing protein so the microbials do not affect any other living organism;
- The toxins do not act on pupae because they do not feed any more;
- The younger the larvae the less toxin they need to digest to die, therefore they usually die quicker than late instars.

Products

Commercially available products
Manufacturer: Valent BioSciences, Illinois, USA

We have to distinguish between two microbials and the two formulations of each microbial that might be used:

Microbials

- Bacillus thuringiensis var. israelensis (VectoBac®)
- Bacillus sphaericus (VectoLex®)

Formulations and application methods

- Water-dispersible Granule (WDG) applied as a liquid with knapsack sprayers
- Corn Granule (CG) applied by hand

Mode of application

Water-dispersible granule (WDG) – diluted in water, applied as liquid with a knapsack sprayer.
Corn granule (CG) – applied as granular, undiluted finished product by hand.

When to use what?

Liquid application with knapsack sprayer:

- Effective and easy to apply in sites that have little emergent or floating vegetation;
- If there is large amount of emergent vegetation the spray may not be able to penetrate the vegetation to enter the water.

Granule application by hand:

- Slower to apply to large areas but broadly applicable, will reach the target in all circumstances;
- Particularly effective in sites with emergent or floating vegetation that liquid applications cannot penetrate;

- Granule penetrates vegetation and drops onto water surface;
- Granule can often be thrown a greater distance than liquid and can therefore be used to treat less accessible sites.

Bti (VectoBac)

- In all habitats, less effective in very polluted habitats (e.g. latrines);
- Needs to be applied weekly;
- Cheap.

Bs (VectoLex)

- In all habitats – including very polluted water;
- Application can give an extended residual effect especially where late instar larvae occur and therefore this needs weekly monitoring;
- Expensive.

Application Dosages

Before the *Bti* and *Bs*. formulations can be used in the field, their actual potency and efficacy has to be evaluated against the different indigenous mosquito species. To assess the minimum effective dosage, bioassays need to be carried out in the laboratory following World Health Organization (WHO) guidelines. To assess the optimum effective dosages, field trials either in natural or in artificial habitats need to be carried out. The outcome of these preliminary tests on larval control answer the following questions: What is the minimum and optimum effective dosage of the formulations against indigenous *Anopheles* and *Culex* mosquitoes? Is *Bti/Bs* suitable for the control of anopheline mosquitoes in the area? Which concentrations have to be used? At what interval does re-treatment need to take place? Which formulations are most powerful? What are the best application methodologies?

Preparatory studies were carried out at ICIPE, Mbita, western Kenya between 2002 and 2004. Following these studies, recommended formulations and dosages for open, potentially *Anopheles*-producing habitats were worked out and are shown in the table below:

To achieve 100% control of mosquito larvae in any habitat in 24 hours, use:

VectoLex WDG (650 ITU/mg)	2.0 kg/ha	0.20 g/m^2
VectoBac WDG (3000 ITU/mg)	0.4 kg/ha	0.04 g/m^2
VectoLex CG (50 ITU/mg)	30 kg/ha	3 g/m^2
VectoBac CG (200 ITU/mg)	10 kg/ha	1 g/m^2

ITU = International Toxic Units, describes the potency of larvicide. The higher the number, the more toxic is the product per 1mg and therefore the less product that is necessary to kill 100% of larvae within 24hrs.

Always note Lot number and ITU of product used in the field. ITU and Lot number are indicated on the product.

Any Bti product (VectoBac) NEEDS to be applied in WEEKLY intervals. Bti products do not have any longer residual effect.

Selection of Larvicide for Dar es Salaam in 2006

We will take two approaches to two different categories of habitats. Open habitats, which are exposed to sunlight and hence potential sources of *Anopheles*, will be treated directly by the programme *Mosquito Control CORPs* with Bti (VectoBac) only. For closed habitats in domestic settings which are not exposed to sunlight and produce no *Anopheles* but lots of nuisance culicine mosquitoes, small amounts of Bs (VectoLex) will be provided to households by programme staff.

Since we deal with highly polluted habitats in the urban area we will double the optimum dosage as identified above for routine use in Dar es Salaam.

For open habitats we will apply:

VectoBac (Bti) CG at 1 gram per square metre (10 kg per hectare)

OR

VectiBac (Bti) WDG at 0.04 gram per square metre (0.4 kg per hectare)

For our first year larviciding we have decided to use only Bti (VectoBac) for open larval habitats. Bti will be applied as corn granule (CG) formulations for hand application and water dispersible granule (WDG) for application as a liquid with knapsack sprayers where this formulation is appropriate. We will use Bti only for open habitats and this product must be applied weekly because it has no residual activity but is the cheapest option and does not require any additional monitoring and decisions on re-application dates.

Application Equipment and Procedures

Liquid application: Solo 475 knapsack sprayers with a capacity of 14 L will be used to apply water dispersible granular (WDG) formulations. They are an effective method of application in sites that have little emergent vegetation. If there is a large amount of emergent vegetation the spray may not penetrate and enter the water. The selected knapsack sprayers are relatively light and simple to use. They use compressed air above the spray mixture to push the mixture out of the tank through a hose and nozzle. The output of the sprayer is dependent on the pressure used, the nozzle type and the speed of walking during the application. Calibration of the knapsack sprayers can be practiced easily following standard operating procedures. The WDG formulations are easy to use since they dissolve in water easily. Therefore, they can be directly mixed in the knapsack sprayer by adding the larvicide and filling the sprayer to its maximum mark. The sprayer needs to be shaken well before pressure is added to the spray mix. To fill a full tank of the Solo 475 sprayer, 400 grams of WDG powder can be dispersed in water by mixing with agitation in approximately half a tank of water (7L), adding the remainder of the water to achieve a total volume of 14L, and then mixing vigorously for 2 minutes. To prepare a half tank, mix 200 g of powder with 3 to 4 litres of water and make up to 7 litres or the halfway mark in a similar fashion. Only when the powder is fully dispersed into liquid form can pressure be applied and application begin: An application pressure of approximately 3 bar is achieved and maintained by pumping a Solo 475 sprayer with a number 2 disk and no core to pressure setting number 3. Calibration in Dar es Salaam indicates a typical mosquito control CORP achieves a swath width of 10 m, a flow rate of 0.74 litres per minute, and a walking rate of 54 m. With this dilution, flow rate, walking speed and swath width, a full tank is expected to cover one full hectare but no more. This is equivalent to 10 x 100 metre swaths across a perfectly square area of one hectare (100 m x 100 m) or 1000 metres of continuous swath. The spray wand should be moved quickly and continuously across a 180° arc using a full swing while walking the length of the swaths.

Calibrated application specifications for liquid application:

- Dilution: 400 g of WDG for a full tank (14L) or 200g for a half tank (7L);
- Backpack configuration: **Number 2 disk with no core**;
- Pressure: Backpack setting number 3 (approximately 3 bar);
- Walking speed: Approximately 50 metres per minute;
- Swath Width: 10 metres;
- Expected usage rate: 1 full tank should treat one hectare or 1000 metres of swath length (eg 10 swaths across a perfectly square 1 hectare area: 100m x 100m). This means that each litre should last for approximately 70 metres of swath length.

Hand application: Granular formulations (CG) may be applied by hand, similar to scattering seeds. However, it takes practice to obtain an even application or maintain the recommended application rate. It is very important for the field staff to practice this exercise well to gain experience in achieving even coverage. For hand application from granular formulation, a bucket is used on a carrying strap to be hung around the shoulders allowing it to rest on the belly. The carrying strap can be adjusted for individual comfort and effectiveness. As determined during a recent calibration workshop, our objective is to achieve a coverage rate of 1 gram of VectoBac CG per square metre (m^2), equivalent to 10 Kg per hectare. For medium to large areas (>9 m^2 or 3 m x 3 m) with multiple habitats, this is best achieved by treating 3 m-wide swaths with one handful spread over 10m of swath length. For smaller, distinct habitats, the area of the habitat should be measured and appropriate fractions of a handful (one handful = 25 g) or a teaspoon (one teaspoon = 2 g) should be applied. For example, for a small habitat of approximately one metre squared, half a teaspoonful should be spread evenly by hand throughout the habitat. For a larger habitat of, for example 12 m^2 (3 m × 4 m), half a handful should be spread evenly across the habitat. For long, narrow (< 1 m) habitats such as remnants of foundation trenches running alongside walls, simply scatter granules in the target area as you walk the length of the habitat, aiming to cover 20–30 m of habitat per handful of granules. For all these habitat types it is best to practice on surfaces where granules are readily seen, aiming to achieve even coverage with approximately 4 granules per 10 cm x 10 cm area. The application specifications for easy reference are summarized as follows:

Calibrated application specifications for liquid application:

- Coverage: Approximately 4 granules per 100 cm^2 or 10 cm x 10 cm area;
- Application rate for small to medium habitats: 1 teaspoon full per 2 m^2;
- Swath width for habitats > 9 m^2 in size: 3 metres;
- Application rate for swaths across habitats > 9 m^2 in size: 1 handful per 10 metres of swath length walked;
- Application rate for long narrow habitats: 1 handful per 20 to 30 m of habitat length.

Evaluation of Larval Control Success

In our study we hypothesize that in comparison to the non-intervention year and the non-intervention sites, controlling the larval stages of mosquitoes in the three intervention wards will result in:

- A smaller proportion of habitats colonized by early instar mosquito stages;
- Late-instar larvae and especially pupae should be rare and extremely difficult to find;
- Far fewer (80% less than otherwise) adult *Anopheles* biting humans;
- Reduced malaria infection and illness in children.

Success depends on:

- Identification of **all** available aquatic habitats within the study area;
- Treatment of **all** aquatic habitats in required dosages (e.g. treatment of drains along their full length);
- Proper performance of the larvicides;
- Treatment at regular weekly intervals so that no late instar larvae are recorded in the sites;
- **No** pupation and emergence takes place in any sites.

Implementation procedures and data recording

Community sensitization

It is mandatory to inform and gain consent from the administration, community leaders and the community members before any larviciding can take place in the intervention areas. Community members are usually very concerned about any pesticide applied by research teams. There is usually the fear that pesticides applied on water could affect human beings or live stock.

District administration officials (and others) need to be visited and informed about the planned activities, and their appearance at community sensitization meetings might be helpful. Community leaders need to be informed, and with their help community meetings need to be held. Any questions and concerns of the community need to be answered to the best of available knowledge. Questions that cannot be answered immediately need to be discussed with the scientists and information brought back to the community. In particular, **families that farm in the intervention areas should be addressed to ensure that information reaches them clearly. These families are likely to be extremely concerned about weekly larviciding and may fear for their crops or animals**. A community information leaflet and a frequently asked questions fact sheet should be distributed during the sensitization meetings.

Community sensitization will be done using various methods, these are:

- Meetings with well known community members/leaders including Ten Cell leaders;
- Public addresses using a megaphone mounted on a car that drives through all the streets just before the intervention;
- Public meetings with the community using traditional ngomas;
- Distribution of leaflets and frequently asked questions at all meetings;
- Availability of larvicides for Household Control of closed habitats (packaging of VectoLex CG);
- Leaflets and announcements to households in the intervention areas directing them to ward office/meeting points to pick up larvicides for mosquito control in pit latrines and other closed habitats.

Field Staff – Mosquito Control CORPs and Larval Mosquito Surveillance CORPs

During the intervention year the weekly larval surveys will be implemented by the *Larval Mosquito Surveillance CORPs* following the same standard procedures as during the baseline data collection. Additionally, in the intervention wards a team of *Mosquito Control CORPs* has been recruited so that surveillance and control of **all** the habitats in the targeted wards are conducted separately. Larval surveillance and application of larvicides will be implemented independently (these two teams of CORPS **do not** cover the area together! Instead, the surveillance team follows, using the same lists of ten cell units two days later).

Mosquito Control CORPs for the three intervention wards for 2006 were recruited in January 2006 and have followed the Mosquito Larval Surveillance CORPs for one month to familiarize themselves with the area of operations. Larviciding will start on March 1 2006. A special timetable has been developed for larval survey CORPs and spraymen specifying days of the week and TCUs to be visited at these days. Spraymen will visit the TCUs first and apply larvicides to all aquatic sites. The CORP will survey the same TCUs one day later for larvae.

Larval Survey Data Recording – Mosquito larval surveillance CORPs

Larval habitat and density data will be recorded weekly in intervention and non-intervention wards following the same procedures and data sheets as for the baseline data collection. All available aquatic habitats will be recorded and larval presence noted. In the intervention wards the larval survey CORP monitors the activity of the sprayman in his/her respective area of responsibility.

If the CORP identifies sites with late instar larvae, he needs to highlight them in the data sheet and report this observation back to the supervisor the same day when he/she brings the data sheets back to the ward office. All larval survey CORPs need to return their data sheets to the ward office after finishing the day's work and inform the supervisor verbally at the same day about any TCUs and sites where old larvae have been found and where larvicide application still needs to be done. The supervisor needs to discuss this with the sprayman responsible for the area.

Larviciding Data Recording – Mosquito Control COPRs

In his area of responsibility (*street* or part of a street) the Mosquito Control CORPs will have to treat **ALL** available sites that contain water at the moment of the visit. This **must** happen weekly and irrespective of the presence or absence of larvae. Therefore, the Mosquito Control CORPs will not carry a dipper and will not record every single habitat that has been treated. The Mosquito Control CORPs searches every TCU that he or she is supposed to visit on this date (following the timetable prepared by supervisors and CMSOs) for any site that contains water (open habitats), also taking into account the experiences gained from following the larval survey CORP during the first 4 weeks of training. **BUT** it is important that the Mosquito Control CORP does not visit only the sites he or she found to have water during training, but finds and treats all potential sites.

Note: The Mosquito Control CORPs are trained during the dry season! As such, he or she will be dealing with more habitats during the rains. Supervisors and Mosquito Control CORPs need to be trained to this effect and CMSOs need to remind them regularly.

The Mosquito Control CORP has to record the following information:

> Week and date of application, TCU visited for larviciding, the total number of TCUs visited, the amount of larvicide received per day (as weight and recorded in data sheet by supervisor), amount of larvicide left after day's work (as weight and recorded in data sheet by supervisor) and the calculated amount of larvicide used per day (calculated and recorded in data sheet by supervisor).

A mosquito control CORP will have one data sheet for every day in the week (Mon, Tue, Wed, Thu, Fri), see example below:

Ward level larviciding - Open habitats

MUNICIPALITY:___Temeke____ WARD:_____Kurasini___ MTAA:_____Kurasini_____
Round (filled at City level):_____

Day	Date	TCU number	Wet habitats present?		Larvicide applied?		Comments
			YES	NO	YES	NO	
MON	01.01.06	001	x		x		
		002	x		x		
		005		x		x	
		007		x		x	
		008		x		x	
		009	x			x	no application because access was denied by residents

Total number of TCUs where larvicide application took place today: .....
Amount of larvicide received today (in kg): .....
Amount of larvicide left (in kg): .....
Amount of larvicide used today (in kg): .....
Mosquito Control CORP signature: ..... Supervisor's Signature: .....

Records on larvicide use and areas treated – Ward Supervisor

The ward supervisors (and the assistant supervisor in Mikocheni) need to keep daily records of the material released and returned per day and need to prepare a weekly summary of used material per Mosquito Control CORP:

- The larvicide will be stored at the ward offices in the intervention wards;
- The ward supervisors will hand out larvicides to the Mosquito Control CORP every morning between 7.00 and 8.00am;

- The released material has to be recorded per Mosquito Control CORP. Both supervisor and Mosquito Control CORPs have to sign;
- A separate material recording sheet will be used for each Mosquito Control CORP and therefore for each area;
- The supervisor weighs the material and indicates the amount released in the his own larvicide release data sheet and in the ward level larviciding data sheet of the Mosquito Control CORP;
- The Mosquito Control CORP returns in the afternoon after finishing the day's work to the ward office;
- The supervisor weighs the remaining amount of larvicides and indicates this in his own and in the Larval Control CORP's data sheet and calculates the amount of larvicide used;
- The larviciding data sheet for the day then remains in the ward office;
- The datasheets need to be checked immediately when they are submitted and, if there is no problem identified, need to be filed in a separate file for larvicide application (1 file per area = 1 Mosquito Control CORP)
- In the case of any problem being identified on the data sheet the ward supervisor **must** discuss this with the larval control CORPs and investigate further. The supervisor needs to discuss the problem with the inspector, and plan and implement appropriate action promptly. In case problems arise that cannot be addressed by the ward supervisor he/she should consult the inspector and, if necessary, the municipal coordinator, immediately. If the problem still cannot be resolved promptly, help should be sought from the City Office **immediately**.

The record of the daily release of larvicides will be completed on one data sheet per Mosquito Control CORPs per month. In this data sheet the supervisor will also indicate how many TCUs have been treated, according to the Mosquito Control CORP, every day. At the end of the week the supervisor calculates the weekly total. This data sheet can then be sent back to the City with the weekly summary records from the larval surveys.

Larvicide Release Records

MUNICIPALITY: WARD: Mtaa: Sprayman's name:

Supervisor's name:

Week:	Day:	Date:	Amount of Granule received (in kg):	Signature Sprayman	Amount of granule returned (in kg)	Amount of granule used	Total number of TCUs treated (as per Mosquito Control CORP data sheet)	Signature Supervisor
1	Mon							
1	Tue							
1	Wed							
1	Thu							
1	Fri							
1	**Weekly Total:**							
2	Mon							
2	Tue							

Culex Control in Closed Habitats

Closed habitats cannot be managed by the spraymen of the programme, who will focus on open habitats only. Given that a large number of closed habitats (latrines, soakage pits, water tanks etc) produce a substantial number of nuisance Culex mosquitoes, the community in the intervention wards might be disappointed if they do not notice a significant reduction in nuisance biting. To increase community support larvicides for treatment of closed habitats for households will be offered free of charge. Small bags of granule will be made available at the area office, on certain dates, for households in the intervention wards. Affected household members can come to pick up the larvicide and a leaflet with directions for use, and can treat the closed habitats themselves. *Bacillus sphaericus* (Bs) granules (CG) will be used for treatment of closed habitats. Bs is very effective in highly polluted water and has a long residual effect in closed habitats. Treatment of closed

habitats has to take place every 2–3 months. One small bag of larvicide will contain 10 grams of granule, which is sufficient to treat up to 10 m^2 of water surface.

Organization: Closed habitat treatment campaigns will be implemented every 3 months in all the intervention wards. The distribution of larvicides for householders will take place on area level at specific dates. Community sensitization will take place a few days before the distribution to inform the community on which date and where they can come to collect larvicides for their closed habitats at household level. The area chairmen will be involved in the release of larvicides to ensure that provision is made only to eligible households members. The householders' names, addresses, type of closed habitats and number of larvicide bags will be recorded per area.

Storage and Distribution of Larvicides

The larvicides will be shipped to the City Office and will be stored at a central store (Kisutu Office). The keys for the store will be handled by City Council staff ONLY. Once a week, the necessary amount of larvicides will be delivered to the ward offices under supervision of the CMSOs. Records will be kept at the central store and at ward level, (account book for in and out needs to be available). Ward supervisors have to sign for the weekly amount of larvicides they receive. The weekly supply will be delivered on Fridays. All ward offices will keep their larvicide stock in a dry and secure place that will be locked and can only be accessed by the ward supervisor. All four sites have been provided with locked cabinets for secure storage of larvicides.

Supervision and Support System for Intervention wards

Inspectors

To support the intervention wards in the first year of larviciding one of the municipal inspectors has been assigned to the priority intervention ward and the non-intervention ward assigned for comparison in each municipality. The inspector will help the ward supervisors with all his/her duties, assist in problem solving, in communications with City Office, and will implement independent spot checks to ensure good quality mosquito control in the intervention wards and data quality in non-intervention wards. Twelve randomly selected spot checks need to be implemented per week: six in the high priority (intervention plus comparison ward) and six in the lower priority (remaining three) wards; the visit of TCUs in the intervention wards need to be implemented 24–48 hrs after a scheduled larvicide application by the sprayman (therefore the inspector has to check the timetable of spraymen and plan which day to carry out a spot check).

Municipal mosquito larval habitat spot check - Open habitats

Serial number of this form _____

Serial number on the map form _____

Ten cell unit identifier _____

Municipality:_____ Ward:_____ Mtaa/area:_____ 10-cell unit:_____ 10-cell leader:_____

GPS(UTM/WGS84): Northing_____ Easting_____ Date of CORP's data sheet:_____/_____/_____

Serial number on the map form_____

	Yes	No
Is there a map?		
Is the map accurate?		
Does map match city copy?		

Habitat codes:

1: Puddles/tire tracks
2: Swampy areas
3: Mangrove Swamp
4: Drain/Ditch
5: Construction pits/foundations/man-made holes
6: Water storage & any other man-made container
7: Rice paddy
8: Matuta
9: Other agriculture
10: Stream/river bed
11: Pond
12: Others (describe below)

Plot ID	House No.	Habitat ID	Habitat type	Is habitat type correct?	Correct habitat type	Habitat found by the CORPs? 1=Yes 2=No	Habitat Description	Wet?		Habitat perimeter			Plants			Water depth		Larval stage Anoph.			Larval stage Culex			Pupae		Comments
								dry	contains water	< 10 m	10-100 m	> 100 m	None	Short vegetation	Tall vegetation / Floating plants	< 0.5 m	> 0.5 m	Absent	Early	Late	Absent	Early	Late	Absent	Present	

Additional targeted spot checks in areas of known larvae production or identified problem areas should be implemented by the inspector and ward supervisor throughout the week. The second municipal inspector will be responsible for the remaining three lower priority wards in the munic-

ipality and will implement his/her routine duties. The routine TCU spot check data sheets remain the same as during the baseline data collection period except for one additional column where the latest larvicide application date (as per timetable of Mosquito Control CORPs) needs to be indicated in the intervention wards.

The results of the additional targeted spot checks in the intervention ward, identified problems and the action taken, need to be included in the inspector's reports.

City Malaria Surveillance Officers:

CMSOs also need to implement independent spot checks in the three intervention wards weekly. Special attention needs to be given to areas where larval habitats are abundant. Spot checks should preferably take place 24–48 hrs after a scheduled application. CMSOs should record the TCUs and habitats (Plot & Habitat ID) visited and the presence or absence of larval stages and pupae. The CMSOs should also enquire whether the spraymen have been seen by the community and record whether any sign of biocide granule (CG) can be seen. A special intervention spot check data sheet (see below) will help to record the observations. This data sheet can be used by CMSOs, inspectors and municipal coordinators. Whenever late instar larvae or pupae can be observed in checked habitats or when complaints from the community are received, immediate action has to be taken (contact ward supervisor, inspector and spraymen, identify the source of the problem, and attempt to resolve this).

Intervention Spot Checks

MUNICIPALITY: _____ INTERVENTION WARD: _____

checked by (name and position): _____

Date	Mtaa	TCU number	Plot ID	Habitat ID	Date of larviciding	Larval stage						Pupae		Sprayman seen?		Signs of CG?		Comments
						Anoph.			Culex									
						Absent	Early	Late	Absent	Early	Late	Absent	Present	Yes	No	Yes	no	

Research Permit

Bti and Bs products are not registered in Tanzania for individual or commercial use. Therefore, an application was made for a research permit from the Tropical Pesticide Research Institute to use these products in the UMCP. Photocopies of the permit should be with all the ward supervisors.

Annex 6

WEEKLY LARVAL SURVEILLANCE SUMMARY FORM

Weekly habitat summary data sheet

Signature Supervisor
Signature Inspector
Signature Co-ordinator

Date: __/__/__
Date: __/__/__
Date: __/__/__

Folder number:

Municipality:
Ward:
Mtaa/area:

Year	Month	Week
2007		

10-cell unit	No. of habitats	No. habitats with water	No. habitats with Anopheles early	No. habitats with Anopheles late	No. habitats with Culex early	No. habitats with Culex late	No. habitats with pupae

10-cell unit	No. of habitats	No. of habitats with water	No. of habitats with Anopheles early	No. of habitats with Anopheles late	No. of habitats with Culex early

Signature:

Annex 7

HABITAT MODIFICATION AND MANIPULATION

This annex addresses habitat modification methods for application to: impoundments, irrigation systems and natural streams; land filling and grading; drainage; and the covering or screening of larval habitats.

For a full list of WHO references relevant to habitat modification and manipulation see (2,3); case studies are available at http://www.who.int/heli/risks/vectors/vectordirectory/en/index1.html. For further information, see also (23,24,25).

1. Habitat modification

1.1 Impoundments

Impoundments are water reservoirs stored behind dams, used for irrigation, drinking water or hydroelectric power.

The construction of a dam will generally reduce mosquito breeding since small larval habitats become amalgamated into one large, deep reservoir which is generally not conducive to breeding, except where water is shallow at the margins or where there is floating vegetation which shields larvae.

A number of design features of dams and reservoirs can reduce the risk of malaria. Dams and reservoirs should ideally be sited away from human habitations. Reservoirs should be sited only where they will not contain a large area of shallow water, since shallow water will be associated with high evaporation rates and drying of the reservoir, potentially leaving behind shallow water conducive to larval habitats. If possible, shallow bays should be deepened, for example via 'cut and fill', as was conducted by the Tennessee Valley Authority (**Fig. 1**). If this is not feasible, then large shallow bays may be isolated using dikes and the land reclaimed through drainage. Small pools at reservoir margins should be drained and vegetation cleared from the sides. Reservoir margins should be as straight as possible to restrict their length. Seepage from the base of the dam should be addressed since this provides larval habitats and wastes water. Off-takes of greater size than normal from the reservoir can be used

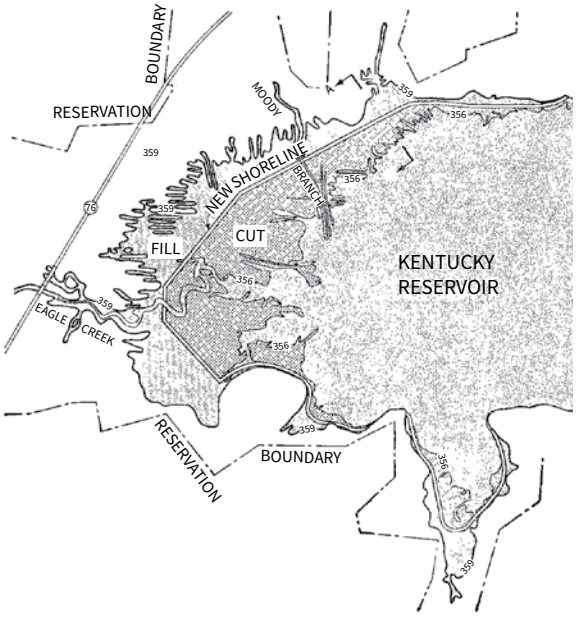


Figure 1. 'Cut and fill' project at Eagle Creek, Kentucky Reservoir, Tennessee Valley Authority TVA, 1947 (*2*)

to rapidly reduce the water level, stranding larvae around its banks. The run-off from this can then be used to flush irrigation channels. Water level fluctuation and stream flushing are covered in the section on habitat manipulation.

1.2 Irrigation systems

Irrigation is the application of water to the ground to maintain soil moisture at levels required for crop production. Poor design of irrigation systems is often associated with larval habitats for malaria vectors, which can be avoided by good design (*2,3*).

Summary of features that can make irrigation systems less conducive to vector breeding (Fig. 2) (*2*):

- Use of safer irrigation methods such as mechanized or localized sprinkler irrigation;
- Use of closed underground pipes rather than open canals to convey water;
- Canal lining;
- Good canal maintenance (e.g. clearance of vegetation so that water flows, or growing bushes over canals to provide shade);
- Intermittent irrigation and periodical drying;
- Canal flushing;
- Grading and levelling of the land to be irrigated;
- Good irrigation practice to prevent over-watering and water accumulating in pools.

In general, the types of irrigation design least likely to facilitate vector breeding are localized sprinkler (trickle or drip) irrigation, sub-irrigation and mechanized sprinkler irrigation. Furrow irrigation is generally preferable to flood irrigation (*2*).

Open earth canals are often used to convey water but may be associated with problems such as seepage and vegetation growth. In addition, if water flow is too sluggish, this will encourage mosquito breeding, while water flowing too quickly will create turbulence, which erodes the canal, leading to silting downstream. Such problems can be avoided by lining irrigation canals with concrete, plastic, membranes (e.g. asphalt) or compressed earth. Lining canals has the following advantages (*2*):

- Reduces seepage which saves water and reduces the amount of standing water for mosquito breeding;
- Increases water flow, flushing aquatic stages away;
- Deters the growth of plants which provide shelter for some vectors.

The provision of stepping stones or bridges may encourage people and domestic cattle not to cross drainage channels or canals and produce hoof or foot prints which may become larval habitats.

To ensure that canals have a sufficiently high elevation to convey water to adjacent canals, their banks are sometimes built up using soil from borrow pits (**Fig. 3**). This has the double disadvantage of encouraging seepage out of the canal, causing standing water to collect, and borrow pits may fill with water and become larval habitats. To avoid this, earth can be borrowed by stripping nearby land that lies at a relatively high elevation (2).

Underground pipes can replace open channels that are not accessible to adult mosquitoes, and have the added advantages of not being restricted by topography and not occupying arable land. A filter system may be required to prevent silt being carried into pipes (*2*).

Irrigation based on land flooding is associated with a high risk of mosquito breeding. Two precautions can help address this (however they are not possible for uncontrolled, 'wild' flooding). Each flood period should not exceed a few days and the area should be dried for at least one day after-

Figure 2. Desirable features of irrigation systems (*2*)

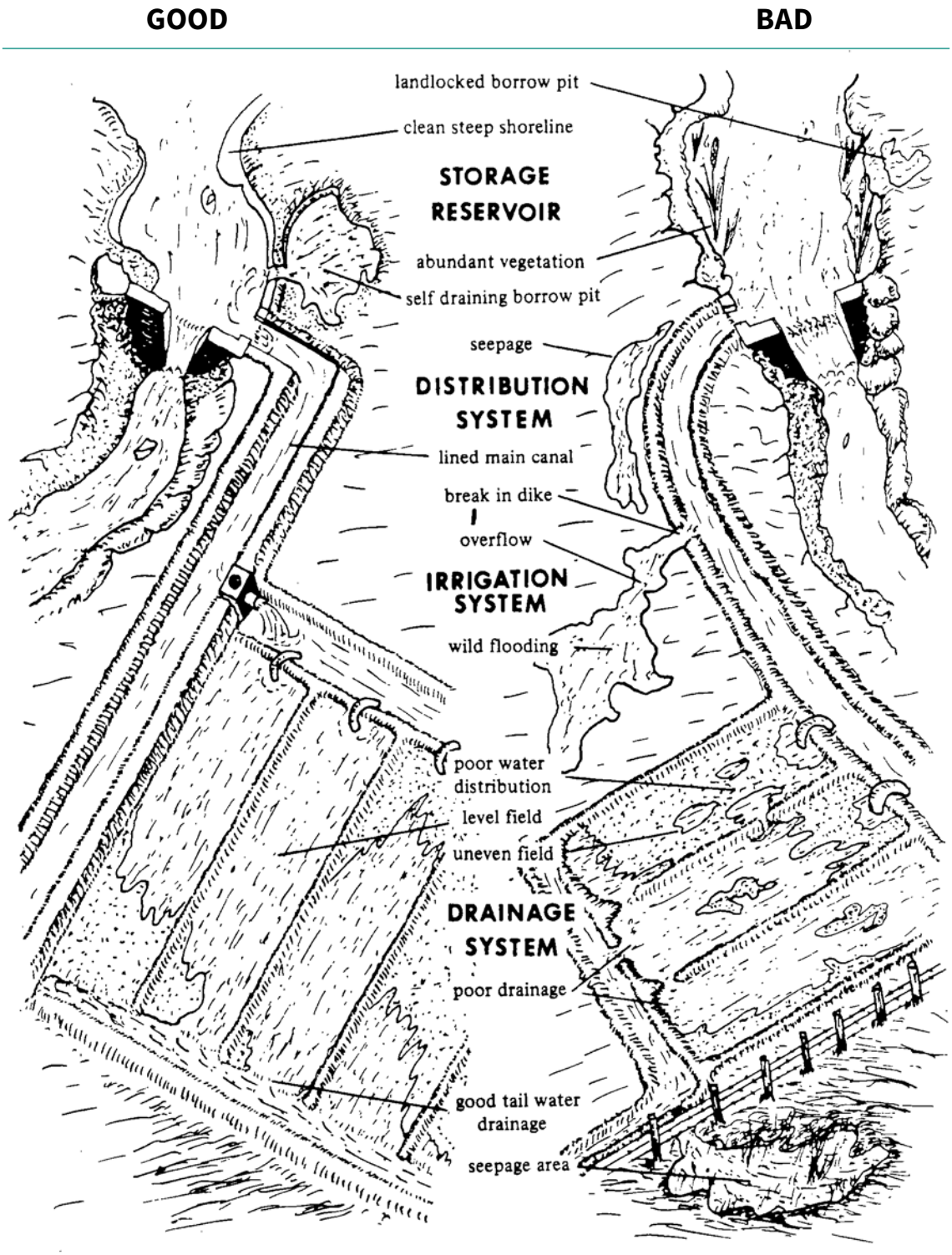
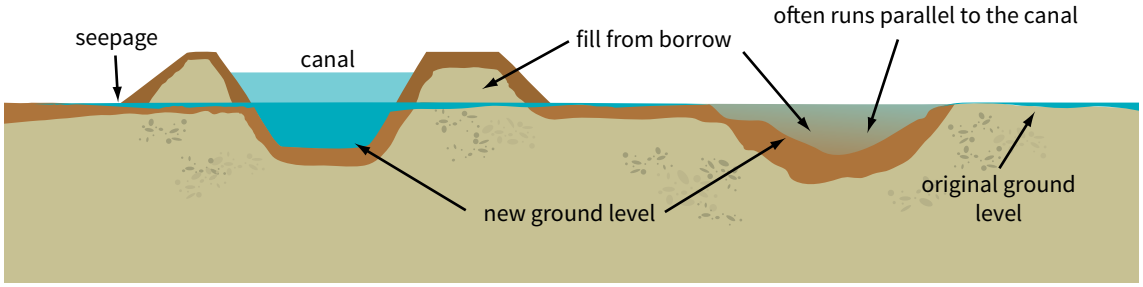


Figure 3. Cross-section of a typical conveyance canal (*2*)



wards. Also, the border strip should be frequently levelled to ensure there are no land depressions, which could fill with water (*2*).

1.3 Natural streams

Backwater pools and isolated seepage pools associated with natural streams are often good larval habitats, in addition to the streams themselves if water flow is sufficiently slow. Channels can be straightened to increase water flow and to reduce bank erosion, floodway channels can be built to relieve natural streams from floodwater, or the central channel can be deepened. The sides of channels can also be strengthened, ideally with gabions (galvanised steel wire mesh cases filled with stones or rocks), which are most robust and flexible, or with solid revetments or retaining walls (*2*).

1.4 Land filling and grading

Mosquito larval habitats such as abandoned ditches, ponds or borrow pits can be permanently removed through filling with soil, rubble, stones, ash or rubbish. No specialist expertise or equipment is required for small-scale works, which communities can conduct themselves. For large-scale works, tractors or diggers may be required. Caution must be taken to avoid creating new larval habitats when collecting filling material (**Fig. 3**). Rubbish can be obtained through collaboration with industrial or public works ministries to save costs. If rubbish is used, it should be compacted and covered with earth to prevent fly breeding. Large areas can be also filled using environmentally safe waste from mining, harbour dredging or demolitions (*3*). Grading to smooth the topography and improve natural drainage is an alternative where the cost of filling is prohibitive or where there is insufficient filling material (**Fig. 4**).

1.5 Drainage

Drainage is the removal of unwanted water on the surface or in the upper layers of soil (**Fig.5**). Good drainage will remove standing water and can be achieved by constructing open ditches with tidal gates, subsoil drainage and pumping (*3*). However, if poorly designed and maintained, the drainage systems used to remove waste water in cities or for agriculture may be important sources of mosquitoes, especially if characterized by leaking, obstructions or small pools of residual standing water.

Summary of features that make drainage systems less conducive to vector breeding (*2*)

- Use of subsoil drains rather than open ditches
- Lining of ditches
- Good alignment of ditches and avoidance of curves
- Ditch flushing and maintenance (see section on habitat manipulation).

Figure 4. Obtaining soil for filling without creating larval habitats (*2*)

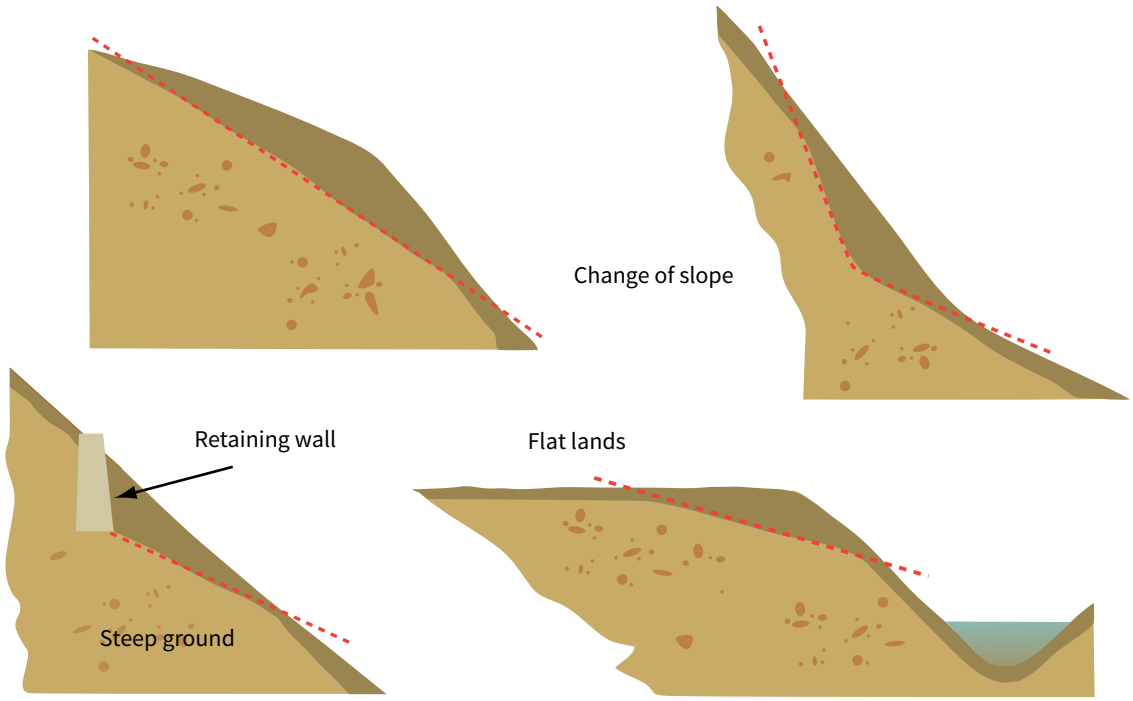
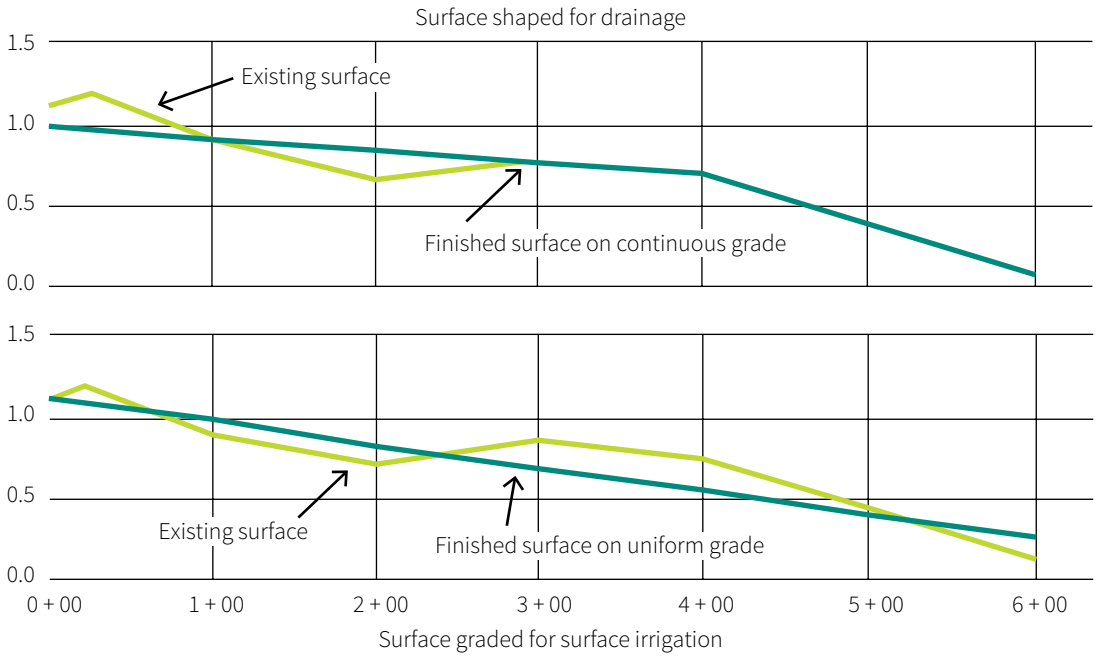


Figure 5. Grading land for drainage and irrigation (*2*)



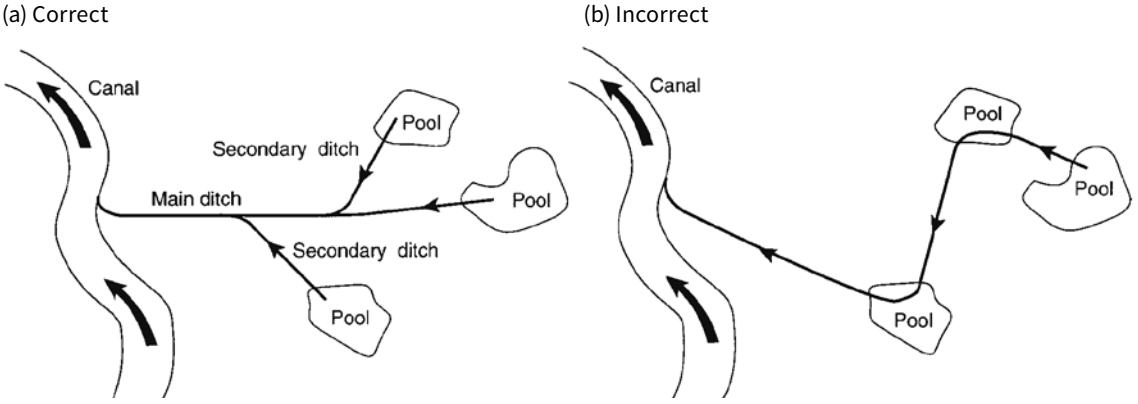
Not only can existing drainage systems be adapted to reduce vector breeding, but in certain settings close to human habitation it may be appropriate to construct new drainage systems specifically for mosquito control. In Zambia, drainage, filling, grading and planting of eucalyptus trees (which dry the soil) was successfully used to control vectors in a public park (*27*).

The design of drainage systems is complex and should be determined by topography, soil type, precipitation, height of the water table, seepage and salinity among other factors. Engineers may be required to help design large-scale drainage systems. However, smaller-scale systems can be constructed by non-specialists with simple equipment (*3,26*), as described below.

Open ditches

Open earth ditches are simple to construct. They prevent excess rainwater accumulating on the ground, and drain pools, marshes or borrow pits. Ditches have a similar structure to irrigation canals despite serving the opposite purpose. They should follow the natural water flow along the land surface to prevent pooling, and lead to a lower-lying outlet (e.g. river, pond, main ditch or soakaway pit) (*3*). Ditches should be short and straight, avoiding sharp bends, to prevent erosion of their banks (**Fig. 6**). Where lateral ditches enter the main ditch, the two water flows should meet at an angle of 30° to prevent erosion of the main ditch bank. The fewer the junctions between ditches the better, since these often become blocked, allowing mosquitoes to breed (*3*).

Figure 6. Correct (a) and incorrect (b) drainage of pools (*3*)



The layout of surface drainage systems is largely determined by the local topography and features such as roads or canals. Typical patterns of drainage systems are shown in **Fig. 7**. Comb, grid-iron and herringbone patterns are the most common layouts for flat, irrigated land (*2*).

A gradient of 1–5 cm per 10 m should give the water enough velocity. Too high a gradient and velocity will lead to erosion of the ditch. The optimal cross-sectional shape of the ditch depends on the soil texture; e.g. vertical sides are appropriate for stiff clay, while sandy soils require ditch sides with a slope of 40 cm horizontally per 10 cm vertically. For most soil, a slope of 10cm:10cm or 20 cm:10 cm is acceptable (*3*). The depth of the ditch should be at least 15 cm lower than the bottom of the water body being drained. Ditch excavation should begin at the downstream end and proceed uphill. Excavated earth can be used to fill in depressions in the ground. It should not be left too close to the ditch where it can be washed into the water, but should be set a little way back, creating a 'spoil bank'. The spoil bank must be perforated to allow water to drain through (**Fig. 8**) (*3*).

Lining ditches with concrete, brick or stone will increase costs but will increase the water flow, reduce plant growth and the build-up of silt, and will be durable and therefore reduce maintenance costs. Lining of earth ditches is essential where rainfall is heavy. Stabilization of banks may be required where the water flow is turbulent, especially where ditches meet. Ditches can be lined

Figure 7. Typical drainage system layouts (*2*)

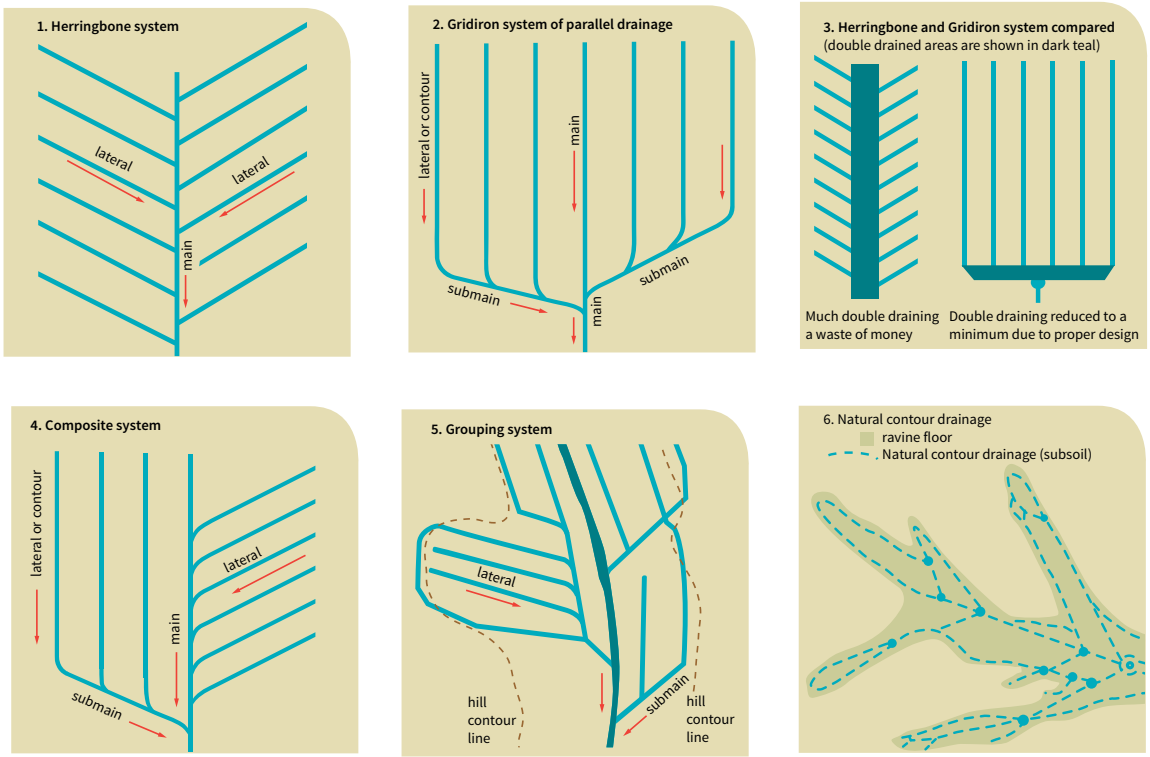


Figure 8. Location and design of spoil banks (*3*)

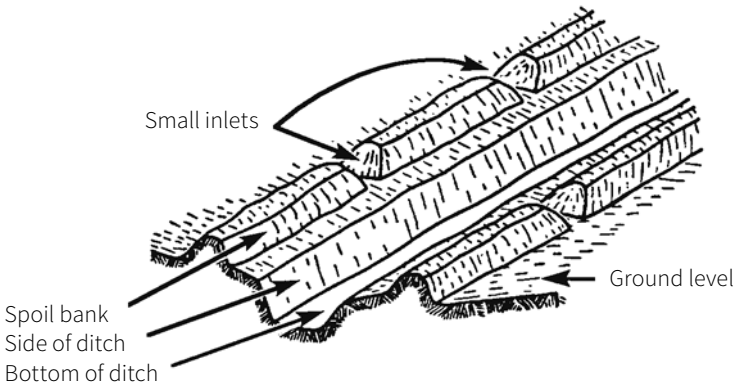


Figure 9. Pre-cast concrete slab for lining ditch (*3*)

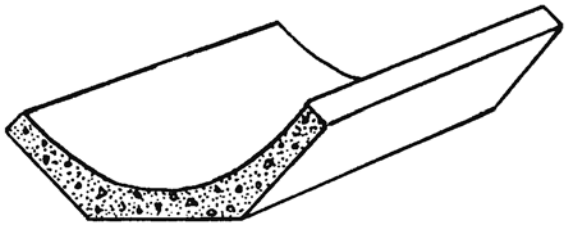
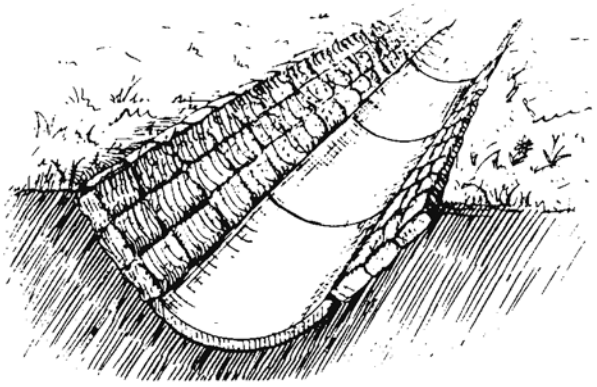


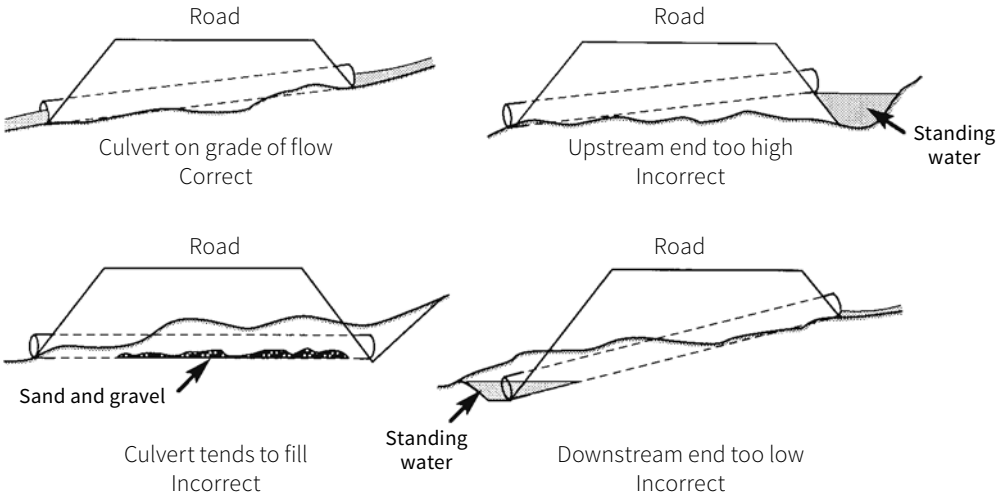
Figure 10. Ditch lined with pre-cast concrete slabs and turf side slabs (*3*)



using flat stones with cement in between, or using a 4–5 cm thick layer of concrete strengthened with wire mesh. Pre-cast concrete slabs (**Fig. 9**) can also be used with turf or concrete side-slabs laid above (**Fig. 10**). Banks should always be kept clear of vegetation (*3*).

Where the ditch narrows under a road or embankment via a culvert or pipe, a large gradient is required to prevent debris accumulating (**Fig. 11**). Culverts can be constructed from wood or concrete or preferably, plastic or corrugated iron. Pipes can be made by cutting the bases off oil drums (*3*).

Figure 11. Culvert design (*3*)

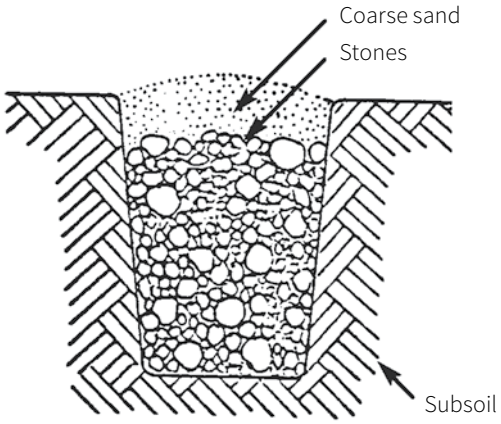


Subsoil drainage

Subsoil drainage is used where the land surface cannot be broken up by ditches or where earth is too unstable for open ditch construction. Expense limits its use in vector control. However its advantages are that refuse or vegetation will not block the water flow, and the addition of larvicides or oils to prevent mosquito breeding is not necessary. Subsoil drainage is often used in irrigated areas and can also be used to lower the groundwater table to prevent collection of surface water (3).

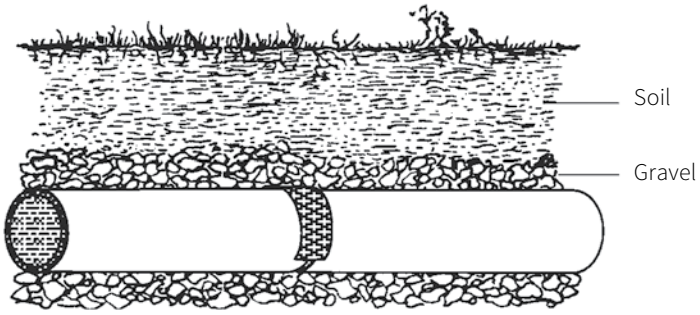
Subsoil drains can be most simply constructed by filling an open ditch with large stones which do not obstruct the water flow, and covering these with leaves, pine needles or sand to prevent silt or clay accumulating at the bottom of the drain (**Fig. 12**) (*3*).

Figure 12. Simple subsoil (French) drain (*5*)



A more sophisticated subsoil drain can be constructed from ceramic tile pipes laid at the base of a ditch 0.5–2 m deep, in an exactly straight line (**Fig. 13**). The joins between pipes are not sealed, allowing water to enter. Silting is reduced by covering the upper surface joins with rubbish, leaves or roofing paper. The ideal gradient is 1:200 to 1:400. Pipes will need to be protected if close to the surface, using small bridges (*3*).

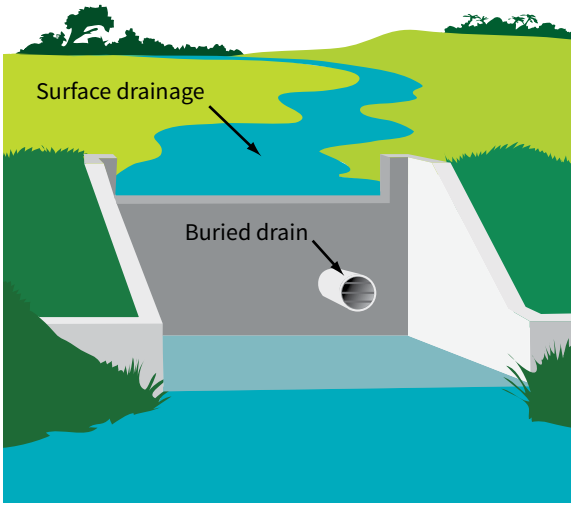
Figure 13. Subsoil (buried conduit) drain constructed from ceramic tile pipes (*3*)



Mole drains can be constructed in cohesive soils, by drawing a bullet-shaped steel former welded to a sharp vertical blade through the soil using a tractor. Mole drains are not permanent and need frequent reforming, however they are effective, and easy and cheap to produce (*3*).

If necessary, both subsoil and surface drainage can be combined (**Fig. 14**). Pump drainage may also be used in some settings, for example where soil has high hydraulic conductivity and water is easily collected in wells these can be emptied by pumping. Alternatively, pumping may be necessary to dispose of water where the topography is not conducive to drainage to the point of disposal (*2*).

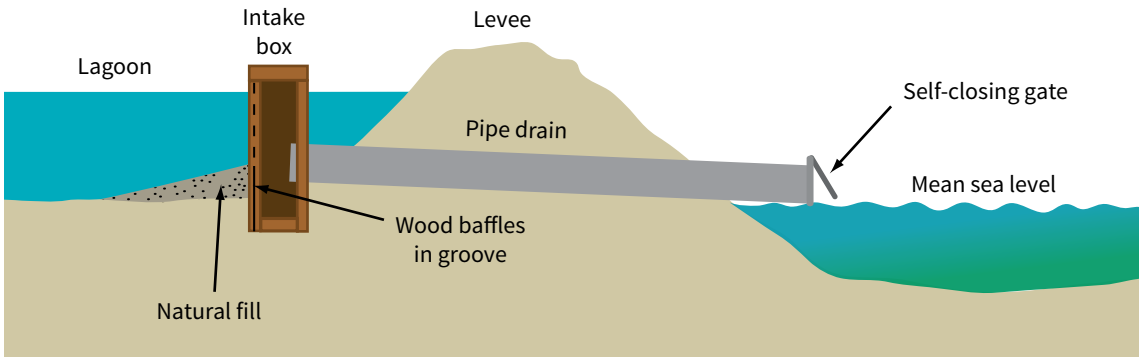
Figure 14. Drop structure for surface drainage and outlet for subsoil drain (*2*)



Coastal swamp drainage

Some vector species breed in brackish coastal marshes, swamps and estuaries. Varying the salinity of these waters can make the habitat unfavourable to anophelines (see section on habitat modification). A more permanent intervention is to construct embankments to aid coastal swamp drainage, by preventing inundation with seawater. Pipes can be fitted in the embankment to allow water to drain at low tide (**Fig. 15**). The upper end of this pipe should be situated near the bed of the lagoon and the lower end should be slightly higher than mean sea level. The pipe should extend some way into the sea, anchored and should end sufficiently high above the sea bed to prevent influx of sand. The pipe should also be fitted with a self-closing gate to prevent inundation of seawater at high tide. Where the lagoon bed is very low, pumps will be needed for drainage (*5*).

Figure 15. Embankment and drain for coastal swamp drainage.
Note the three-walled concrete intake structure, into which boards of 15–20 cm width may be slid, to adjust the sill height of the opening as the lagoon naturally fills with silt (*2*).



If it is not possible to construct an embankment and drain, an alternate system is to construct ditches that connect all low tide pools with the sea, to ensure continuous inundation with seawater and to maintain salinity at a sufficiently high level to prevent mosquito breeding.

Vertical drainage

Where land is too flat to allow water to flow, and in silt or clay areas liable to flood, shafts can be constructed through impermeable strata if there are permeable bedrock strata below, to allow water to drain through. Shafts will remain effective for longer if protected by casing or filled with stones, gravel and coarse sand (*2*).

The 'Lido system'

Where drainage is difficult due to an abundance of vegetation, deepening the water body can prevent plant growth. Banks can be steepened and larvivorous fish introduced to control the larval population (*28*).

Eucalyptus trees

Marshy areas and land with a high water table can be drained by planting eucalyptus trees, the leaves of which allow water to evaporate rapidly.

2. Habitat manipulation

Habitat manipulation is a form of environmental management aimed at producing temporary conditions that are unfavourable to breeding of vectors. Unlike habitat modification, habitat manipulation must be repeated to remain efficacious, and is normally directed at one particular vector species.

This section addresses the following methods of habitat manipulation: controlling water levels (including intermittent irrigation), stream flushing, shading, clearing of aquatic vegetation, straightening and steepening of shorelines, changes to water salinity and water pollution.

2.1 Controlling water levels

Fluctuations in the water level in impoundments or irrigation systems lower vector breeding by (i) discouraging the growth of plants which provide shelter for larvae along margins, (ii) removing larvae from vegetation at margins so they are more exposed to predators and water turbulence, and (iii) stranding larvae at margins. The interval between fluctuations must be shorter than the life of larvae (7–10 days) and the water level should vary by 30 to 40 cm (*3*).

In the tropics, floating vegetation may undermine the effectiveness of managing water levels for controlling mosquito breeding, such as the water hyacinth (*Eichhornia* spp), water chestnut (*Trapa* spp), water primrose (*Jussiaea* spp), water lettuce (*Pistia* spp), alligator weed (*Alternanthera* spp), water milfoil (*Myriophyllum* spp) and Salvinea spp (*2*). Where they are abundant, species such as these will require control, as discussed later in this section.

Intermittent irrigation

In rice-growing areas, intermittent irrigation can control vector breeding and is a legal requirement in some countries. If the ground is level with good drainage, paddy fields can be completely dried for 2–3 days at regular intervals. Drying must occur simultaneously across a large area of farmland. This is not possible in the three weeks after first transplanting rice seedlings and during this period other methods of larval control will be required. The length of time intervals between drying will need to be determined by an expert. Intermittent irrigation may increase yields by suppressing growth of weeds (*3*).

Intermittent irrigation has successfully controlled malaria in India, China and other areas of South-East Asia. In Sichuan Province, China, fields are flooded for a maximum of 100 days per year during the rice-growing season in the summer, and during the winter 'dry' crops such as wheat or cash crops are grown. In India, intermittent irrigation has been combined with the application of neem tree (*Azadirachta indica*) extracts. Intermittent irrigation is less successful for the control of mosquitoes such as *An. gambiae* s.l. which rapidly re-colonize larval habitats after flooding and also flourish in small residual puddles left after drying. In Sri Lanka, it is necessary to maintain water at a constant low depth to prevent pooling and the breeding of *An. culicifacies*. The local vector needs to be assessed however, because in some settings a continuous flow of water is conducive to the breeding of certain species (*28*).

2.2 Stream flushing or sluicing

Flushing is used for small streams with a sufficiently slow and continuous flow of water to allow mosquitoes to breed along margins. Periodic flushing with a large volume of water washes away eggs, larvae and pupae from the banks, or strands them at a higher level on dry land. Flushing also stirs up sediment at the bottom of the stream which can bury aquatic mosquito stages and can help slow the growth of new marginal vegetation. Despite the high initial investment required, flushing is a long-lasting method, which requires little maintenance (*5*). It has been successfully used in South-East Asia to control *An. maculatus* and *An. minimus*. It is less appropriate for species that do not prefer streams or where water is in short supply (*28*).

A small dam should be constructed to collect the water required for flushing, at a point where the stream is narrow and banks are high. A sluice gate built into the dam can be opened once a week. Flushing should begin at the start of the mosquito breeding season and end when the stream has dried up (*3*).

2.3 Shading

Planting trees or shrubs along the banks of streams can control mosquitoes, which prefer to breed in partial or full sunlight. This method has been successfully deployed in Assam, India, to control *An. maculatus*. This method may also be suitable for control of *An. fluviatilis*, *An.sundaicus* and *An. minimus* (*2*). Harvesting of black mangrove (*Avicennia* spp) trees from coastal swamps allows new emergent vegetation to grow, which can lead to the proliferation of the African vector *An. melas*. Planting new mangroves will shade the water and deter breeding (*2*). This, however, may not be practical under normal conditions.

2.4 Clearing of aquatic vegetation and algal mats

Some mosquito species may be controlled by clearing water vegetation, since this removes shelter for larvae. This can be easily done using rakes in small larval habitats and in larger larval habitats by applying herbicides or adding herbivorous fish such as the grass carp. This may be impractical in some settings such as swamp forests. Driftwood should also be removed from larger water bodies such as reservoirs (*3*).

2.5 Straightening and steepening shorelines

Straightening and steepening the margins of ditches, streams and ponds removes shallow water suitable for vector breeding, and increases the water flow which washes away eggs, larvae and pupae.

2.6 Changes to water salinity

Vectors which breed in brackish waters in coastal marshes or lagoons may be controlled by introducing seawater into their larval habitats via sluice gates, culverts or channels to increase water salinity. This is feasible only where the vector species in question does not tolerate salt water and where rainfall is not excessively heavy. This method has been successfully used to control *An. farauti* in areas around Honiara in the Solomon Islands (*28*). Other species that can be targeted with this method include: *An. sundaicus* in Asia; *An. melas* and *An. merus* in Africa; *An. labranchiae*, *An. antroparvus*, and *An. sacharovi* in the Mediterranean region; and *An. albimanus*, *An. aquasalis* and *An. grabhamii* in the Americas (*2*). Good knowledge of the salt tolerance of the local vector is required before deciding to use this form of vector control.

Larval Source Management (LSM) in Dar es Salaam, Tanzania

A prominent example of LSM in urban sub-Saharan Africa is the **Urban Malaria Control Program** (UMCP), Dar es Salaam, initiated by the City Council in collaboration with Ifakara Health Institute and overseas academic institutions [1-2]. In 2004, mosquito surveillance systems were established and after one year of intensive baseline data collection (2005-2006), operational larviciding with *Bacillus thuringiensis var. israelensis* commenced in three wards during March 2006 [2-3]. In 2007 the intervention was expanded to nine urban wards. Since 2008, 15 wards comprising 56 km^2 and 614,000 city residents have been covered.

Background

- **Site characteristics:** Dar es Salaam is a coastal city with a population of about 2.5m and a total administrative area of 1,393km^2. LSM was introduced into three municipalities (Kinondoni, Temeke and Ilala) which are divided into wards and sub-divided into 360 neighbourhoods (mitaa) [2-3].
- **Climate**: Hot and humid, with long rains from March to May and short rains in November-December. Average annual rainfall is 1115mm [4].
- **Primary and secondary vectors**: The primary vectors are *Anopheles gambiae* and *An. funestus*. *An. merus* is a secondary vector.
- **Main types of breeding site:** *An. gambiae* largely breeds in clean freshwater, but can be found in nearly every type of water, including polluted water bodies; *An. funestus* breeds in permanent water bodies such as inland marshes [4]. Over 70% of larval habitats in the city are man-made, of which many are drains [2].
- **Malaria transmission:** Transmission is low and perennial and 90% of cases are *Plasmodium falciparum* [4]. Outdoor biting is common, possibly because mosquitoes find it difficult to enter houses with closely fitted doors and windows, as well as the high bed net coverage. Urbanisation has generally reduced breeding sites [4] however shanty towns at the periphery of the city are characterised by open sand and borrow pits which are ideal breeding sites [4].

The larval source management program

- **Structure of the control program:** The Urban Malaria Control Program is fully integrated into the Dar es Salaam City Council administrative system and operates at five administrative levels: the City Council, municipalities, wards, neighbourhoods and over 3000 Ten Cell Units (TCUs) [5]. Community-Owned Resource Persons (CORPS), modestly paid members of the community, are responsible for routine mosquito control and surveillance and report to Ward Offices. All standard operating procedures and forms are available online [2].

Figure 1. *Dar es Salaam*

- **Baseline mapping and data collection:** Baseline mapping of all targeted areas was conducted in 2004. Each CORP was then allocated a small area (approximately 0.6km^2), in which they were responsible for larval surveillance. All larval habitats were mapped, classified into one of twelve categories and checked once a week for the presence of larvae using up to 10 dips with a 350ml dipper [6]. Anophelines and culicines were differentiated and larvae classified as early or late instar. In the first year (2004), over 65,000 potential *Anopheles* habitats were surveyed by 90 CORPs every week [2].
- **Larviciding:** Following one year of baseline data collection by the CORPs, one ward per municipality (three in total with a combined area of 17km^2 and 128,000 residents) was selected for intensive surveillance and larval control, based on staff competence. One non-intervention 'comparison' ward from each of the three municipalities was selected for intensive surveillance, using the same criteria as the intervention wards. Surveillance was also continued and improved in remaining wards [2]. Larviciding commenced in March 2006 with *Bacillus thuringiensis* var. *israelensis* strain AM65-52 (*Bti*; VectoBac®, Valent BioSciences Corporation (VBC), USA) and *Bacillus sphaericus* strain 2362 (*Bs*; VectoLex®, VBC), applied as two formulations: (1) water-dispersible granules

suspended in water and applied using Solo® 475 knapsack sprayers and (2) corn granules applied by hand [2]. The program targets culicine mosquitoes in addition to anophelines to reduce nuisance biting and maintain community support [2]. 'Open' habitats exposed to sunlight are treated weekly by Mosquito Control CORPS, and shaded 'closed' habitats (e.g. pit latrines) are treated every three months. Insecticide stocks are managed at a central storage location [2]. Daily insecticide use is recorded by each ward supervisor and monitored at city-level. In May 2007 the program was expanded to nine wards and then to 15 wards in March 2008 [5].

***Figure 2**.Dipping for larvae*

- **Ongoing larval surveillance:** During the early years of the program, 90 Larval Surveillance CORPs were deployed at any given time, each responsible for surveying open habitats in assigned Ten Cell Units (TCUs, the smallest administrative area in the city) down to plot level the day after treatment and reporting to the Ward Office. A plot here refers to a housing compound or small area of land. Municipal Mosquito Control Inspectors (MMCIs) independently validated the work of the CORPs via twice weekly spot checks [2-3]. Larval surveillance data were collated by Ward Supervisors and forwarded to the Municipal Mosquito Control Coordinator (MMCC), who submitted monthly reports to the City Mosquito Control Coordinator (CMCC). Recently this system was updated and larval surveillance is now conducted directly by Ward Supervisors, who visit six TCUs per week selected by the program managers and six further TCUs chosen at their own discretion. Daily reports are uploaded using mobile phones to a web-based server and made available through a password protected link. It is envisaged that this will improve access to data and dramatically reduce operational costs.
- **Adult mosquito surveillance:** Initially, Mbita-design bed net traps, CDC light traps and pyrethrum spray catch were shown to be ineffective at catching *Anopheles* species, possibly because indoor biting is rare. Therefore human landing catches (HLC) were used as a pilot method, with 67 CORPs surveying 268 locations monthly. However this was costly, difficult to sustain and exposed workers to potentially infectious mosquito bites [2]. Therefore an intensive community-based system for routine surveillance using the Ifakara Tent Trap (ITT-C) was developed and implemented in the 15 UMCP wards in February 2009 and 16 adjacent non-intervention wards in October 2009, covering an area of 160km^2 and 2.65 million residents [7]. One person per ward was recruited to conduct monthly night-long surveys in 20 locations per ward and modestly remunerated with US$2.70 (2010) per trapping night. Caught mosquitoes are identified in a central laboratory to the genus level and anophelines identified to species level. *Anopheles gambiae* mosquitoes are identified to sibling species level by PCR. ELISA is used to determine whether mosquitoes are infected with sporozoites [7]. ITT-C has been evaluated by two quality assurance (QA) teams who in turn conducted ITT-C and HLC at randomly selected locations. This showed that ITT-C had lower sensitivity than HLC, however was cost-effective and could predict the odds of human parasite infection [7].
- **Funding:** Government of Tanzania; Bill and Melinda Gates Foundation; United States Agency for International Development; United States President's Malaria Initiative; Valent BioSciences Corporation; Swiss Tropical and Public Health Institute; Wellcome Trust; Swiss National Centre of Competence in Research (NCCR) North-South; Innovative Vector Control Consortium and European Union through AvecNet (African Vector Control New Tools).
- **Other malaria control interventions:** As part of the Malaria Control Strategic Plan 2008-2013, Long Lasting Insecticidal Nets (LLINs) are distributed through a voucher scheme. 70% households owned at least one ITN in 2007-2008 [8] and 62% prior to LLIN distribution in 2010 [9].

Impact

Clinical outcomes: malaria infection prevalence in children aged 0-5 years declined between baseline data collection in 2004-2006 and after the introduction of larviciding in 2006-2007 (Odds

Ratio = 0.284, 95%CI 0.101-0.801; adjusted for location, survey round, LLIN use and repellent use; Fig. 3) [3]. It is difficult to directly attribute the decline in malaria to larviciding. Certainly, there is little evidence that larviciding had any impact on the prevalence of malaria infection among all age groups [3]. Figure 3 also indicates an interesting decline in parasite prevalence in non-intervention wards. However, post-intervention, parasite prevalence is significantly lower in intervention wards in children aged 0-5 years (Fig. 3).

- **Entomological outcomes:** malaria transmission declined between April 2005 and March 2007 (crude relative annual Entomological Inoculation Rate = 0.683, 95%CI 0.491-0.952) alongside a significant reduction in malaria vector abundance and biting rates [3].
- **Effect of other interventions:** Other interventions such as house screening, LLINs and the introduction of artemisinin combination therapies may have also contributed to the decline in malaria [5].

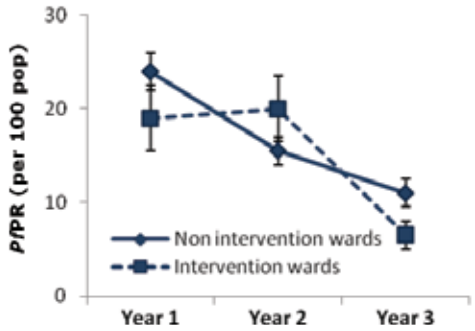


Figure 3. *Crude malaria infection prevalence in children aged 0-5 years during April 2004-March 2005 (year 1, pre-intervention), April 2005-March 2004 (year 2, pre-intervention) and April 2006-March 2007 (year 3, intervention). Error bars show 95% CI [3].*

Factors contributing to success

- Public involvement.
- The LSM program was initiated at 'grassroots level' by Ilala Municipality, which then evolved into the Urban Malaria Control Program which was supported by academic institutions and the government [10].
- Surveillance systems were built up slowly to achieve the standard required [2]. LSM programs learn from previous years of experience and improve.
- Decentralized management [2].
- Drain maintenance contributes substantially to the reduction of larval breeding sites [1].

Challenges

- Some residents do not allow larviciding teams to access habitats in their compounds [3, 5].
- Weekly treatment of breeding sites is required because the larvicides used have low residual efficacy [5].
- All mosquito species must be targeted to reduce nuisance biting and maintain community support.
- Close supervision of larviciding teams and continuous monitoring is required [5].
- Achieving sustainability is an ongoing challenge. International donors handed funding of the program to the Tanzanian Government in 2010. Before this, implementation costs had to be halved from <$1.00 to <$0.50 per person per year [5, 12].
- Concurrent malaria control interventions complicate the measurement of the impact of LSM [5].

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Acknowledgements

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Larval Source Management in Khartoum, Sudan

Malaria was the major cause of outpatient attendances, admissions and deaths in Khartoum in the 1980s and 1990s. This led to the launch of the **Khartoum Malaria Free Initiative** (KMFI) in 2002 by the State and Federal Ministry of Health, in collaboration with the World Health Organization (WHO), with the aim of reducing malaria incidence in Khartoum State by 80% between 2002 and 2008, to less than 0.5 cases per 1000 people per annum [1] and to demonstrate the potential of modern malaria control interventions in order to attract funding for malaria control in the rest of the country [2]. The KMFI has three main components: (1) diagnosis & treatment, (2) prevention and (3) epidemic surveillance [2]. Larval source management is an essential component of the malaria prevention program. This document outlines the structure of the KMFI and its impact.

Background

- **Site characteristics:** Khartoum state is located on the southern fringes of the Sahara, at the junction of the Blue Nile, White Nile and River Nile, 400m above sea level (Fig. 1). The state consists of seven localities [3].
- **Climate**: There are four seasons: winter (mid-November-March); a hot, dry summer (March-July); a rainy season (July-September) and a short, hot transitional season (September-November). Temperatures ranges from 12-45°C and average annual rainfall is 110-200mm [4].

Figure 1. *Khartoum state*

- **Primary vector**: *Anopheles arabiensis.*
- **Main type of breeding sites:** Irrigation canals, pools created from leakage of water pipes, water basins, storage tanks, rain pools and river bed pools [2].
- **Malaria transmission:** Transmission is low and seasonal. *Plasmodium falciparum* accounts for 95% cases. *Plasmodium vivax* and *P. ovale* are also prevalent.

The larval source management program

Structure of the control program: The Khartoum Malaria Free Initiative (KMFI) divides Khartoum State into 7 localities, which are further divided into 26 administrative units, then sectors (areas of 7-10km^2 with a one week working load) and finally sub-sectors (areas of 1-1.5km^2 with a one day working load (six per sector)). The program aims to protect a population of 2,073,300 in urban areas, 3,201,021 in peri-urban areas and 640,672 in rural areas [3]. The KMFI employs 14 trained medical entomologists, 60 public health officers, 31 sanitary overseers and squad leaders, 360 assistant sanitary overseers and 705 spray men [3]. Each public health officer is supported by the sanitary overseers and assistant sanitary overseers and supplied with the necessary equipment [1]. In 2002, WHO commissioned experts from the Oman malaria elimination program to give the program technical support [1].

- **Baseline mapping:** Both potential and actual vector breeding sites have been identified and mapped and target areas classified into the following epidemiological zones: urban, peri-urban, rural riverine, and rural non-riverine (pastoral) [2, 5].
- **Larval source management:**
  - ***Repair of broken water pipes and the removal of water basins by law*** (Fig. 2): the KMFI collaborates with the Water Corporation Department (WCD) to repair broken water pipes. KMFI is responsible for surveillance, reporting and transportation and the WCD provides engineers and equipment. By 2004,

3,818 metres water pipes had been replaced and 6,104m repaired [2].

Figure 2. *Removal of water basins (photo courtesy of KMFI)*

- ***Intermittent irrigation****:* regular drying of irrigated fields, which reduces vector breeding, is compulsory in both government and private irrigation schemes. This initiative is supported by the Farmers Union and the Ministry of Agriculture. 98.2% irrigation schemes were dried for at least 24 hours during 2011 [3]. Leakages from irrigation canals are also repaired and vegetation around canals is cleared in conjunction with the Ministries of Irrigation and Agriculture [2].
- ***Larviciding with temephos:*** In addition to the KMFI workforce, 405 schools and 287,000 pupils are involved in treating breeding sites with temephos [3].
- ***Biological control with*** **Gambusia** ***fish:*** *Gambusia* fish have been added to 317 permanent breeding sites, mainly irrigated canals and pools from leakage of drinking water pipes [3].

- **Public involvement**: Efforts to involve the public in the KMFI have been intense (Fig. 4), through the distribution of information leaflets, regular radio broadcasts and television coverage, health education in schools in collaboration with the Ministry of Education, the organisation of an annual 'Khartoum State Malaria Day', public meetings and the establishment of malaria control committees and societies [2].
- **Clinical surveillance:** Since 1998, annual cross-sectional surveys have been undertaken in random samples of residential blocks every September. Between 1998 and 2009, 256 clusters across 203 samples were surveyed; a total of 128,510 slide examinations [6].
- **Entomological surveillance:** Fortnightly entomological surveys are conducted at 24 sentinel stations by a medical entomologist and entomology technicians. Data are compiled and reported to localities and the state malaria control program using a standard form [2].
  - **Larval surveillance:** At each sentinel site, all known breeding sites within the vicinity are sampled, with 3-10 dips taken with a 0.5L dipper. The average number of larvae per dip is calculated. Larvae are classified according to stage and species. In 2004, 12,360,284 breeding sites in Khartoum State were inspected by 705 mosquito collectors [2].
  - **Adult mosquito surveillance:** Pyrethrum spray catches are conducted in seven rooms at each sentinel site, between 6.00am and 8.00am, and the average number of anophelines per room per station calculated. All mosquitoes collected are classified according to physiological status as unfed, fed, half gravid and gravid [2].
- **Funding:** Government of Sudan; United Nations. The annual cost of the KMFI in Khartoum State is US$0.10 per person protected per annum [3].
- **Other malaria control interventions:** Strengthening of case management through the improvement of microscopy, staff training and provision of antimalarial drugs through the 'revolving drugs fund' [1]. Indoor residual spraying (IRS) and long-lasting insecticidal net (LLIN) distribution are not conducted in Khartoum, however LLINs are exempt from import tax in order to encourage private sector sales [2]. Nonetheless, in the northern states of Sudan, the national malaria control program distributed nearly 0.6 million LLINS between 2006 and 2009 [7].

Impact

Clinical outcomes: Overall parasite prevalence increased from 2.5% in 1999 to 3.2% in 2000 and fell to <1% in all subsequent years to 2009. >90% of all surveyed clusters reported no infections between 2006 and 2009. However notable clusters of malaria infection remained in 2009 at the confluence of the White and Blue Nile rivers [6]. Total confirmed and unconfirmed malaria

cases, as a proportion of total outpatient attendances, declined from 40% in the 1990s to <20% in 2004 (however diagnosis simultaneously improved) [1]. Total malaria deaths (confirmed and unconfirmed) declined by almost 75% from 1,070 in 1999 to 274 in 2004 [1].

It is not possible to directly attribute the decline in malaria to the KMFI, however there is a strong temporal association between the two. It is unlikely that changes in rainfall can explain the trend [6]. Data on the distribution and coverage of KMFI control activities is not available at a sufficiently fine spatial scale to allow attribution to changes in parasite prevalence recorded with the cross-sectional surveys. Travel history data were not collected during these surveys, so local and imported cases cannot be distinguished [6].

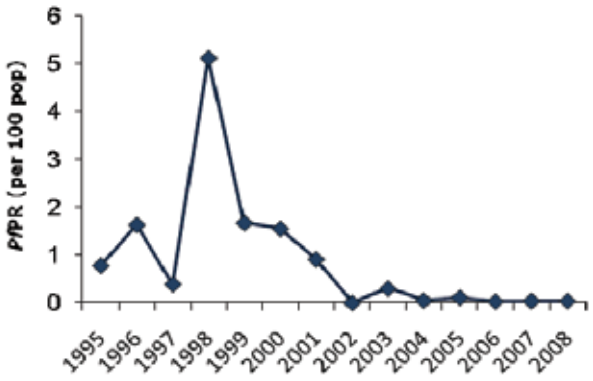

Figure 3. Plasmodium falciparum *parasite rates (PfPR) (all ages) between 1995 and 2008 in Khartoum. No surveys were conducted in 2002 [1-2].*

Entomological outcomes: The total number of breeding sites under surveillance increased from 1,854,856 to 12,360,284 between 2001 and 2004. The percentage of breeding sites producing anopheline larvae and the overall mean larval density did not alter between 2001 and 2004. The average density of adult mosquitoes per room recorded declined from 1.6 mosquitoes/room in July 2001 to 0.4 mosquitoes/room in July 2004 [1].

Factors contributing to success

- Public support is high and KMFI has a high profile due to good public relations [2].
- Technical support from the Oman malaria elimination program [1].
- Strong political support for the control program at both State and Federal level [2].
- Collaboration between the Ministry of Health and the Ministries of Education, Water Corporations & Agriculture; private sector involvement.

Challenges

- Sustaining funding [2].
- Improving entomological surveillance [1,6].
- New agricultural schemes and new construction sites create more breeding sites [3].
- Emergence of *P. vivax* in parts of Khartoum State [3].
- Two decades of conflict have weakened the health system.

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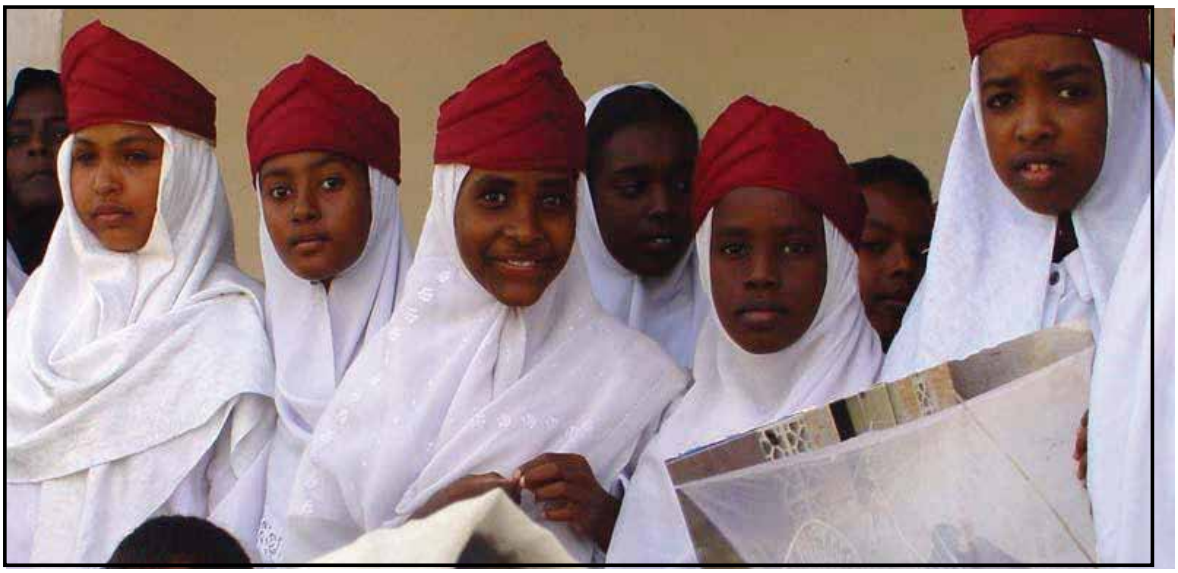

Figure 4. *Raising public awareness of the Malaria Free Initiative (Photo courtesy of KMFI)*

Larval Source Management (LSM) in Mauritius

Malaria was first introduced to the island of Mauritius from mainland Africa during the mid 19th century [1]. Over a century of malaria control campaigns ensued, including large indoor residual spraying (IRS) operations and widespread larval source management (LSM) projects [2]. Mauritius was declared malaria-free in 1973 by the World Health Organization [3]. However, after a series of cyclones from 1975 to 1976, the country saw a resurgence of *Plasmodium vivax* when migrant workers from malaria endemic countries arrived on the island to repair the damage caused by these cyclones. Once again, the country assumed a control campaign with IRS, larviciding and robust surveillance. Since 1997 no indigenous cases of malaria have been reported in Mauritius and the country is now in the 'prevention of reintroduction' phase [4]. LSM remains a mainstay of the **Prevention of Reintroduction Program (PRP),** along with a strong surveillance system and a passenger screening program.

Background

- **Site characteristics:** Mauritius is an island situated in the Indian Ocean, 850km to the east of Madagascar (Fig. 1).
- **Climate**: There are two well-marked seasons: summer and winter. Summer is generally accompanied by heavy showers and occasional cyclones while dry or semi-dry conditions prevail in winter. Mean annual temperature varies with seasons and altitudes, ranging from 21-23°C in summer in the coastal region, and between 16-18°C in winter. Annual rainfall varies from 600-1900mm near the coast to 2500-4450mm in the uplands. The true rainy season extends from December to April.
- **Primary and secondary vectors**: *Anopheles funestus* and *An. arabiensis* were responsible for malaria epidemics between the mid 19th and mid 20th centuries [2]. In 1948, a vector control scheme based on DDT IRS led to the elimination of *An. funestus* from Mauritius by 1950, with a subsequent fall in the incidence of malaria. This scheme failed to control *An. arabiensis* however, which has never been eliminated from the island.

Figure 1. *Mauritius*

- **Main type of breeding sites:** *Anopheles gambiae* mainly breeds in clean, fresh sunlit water bodies.
- **Malaria transmission:** No autochthonous cases of malaria have been reported in Mauritius since 1997 however imported malaria cases among visiting or working expatriates and residents returning from malaria endemic countries still occur, with 54 imported cases in 2011.

The larval source management program

- **Structure of the control program:** In 2009 the Integrated Vector Management (IVM) Concept was introduced and the public is now involved in reducing larval breeding sites through environmental modification (e.g. maintenance of drains and storm drains and management of solid and bulky waste), which reduces dependence on larvicides. Members of the community are educated by health inspectors who have Power of Entry granted by the 1925 Public Health Act, according to which it is a legal requirement for individuals to remove breeding sites around their homes [5]. Routine larviciding is also conducted island-wide.
- **Baseline mapping and data collection:** All breeding sites in target areas are mapped and new breeding sites are detected during routine surveys once a month.
- **Larviciding:** Former foci of malaria transmission, highly productive breeding sites identified through entomological surveillance and standing water within a 500m radius of the residences of imported cases and migrant workers are treated fortnightly with temephos (Fig 2). In addition,

Bacillus thuringiensis var. *israelensis* (Bti) is currently applied in nine villages in 40 to 50 housing units.

- **Larval and adult surveillance:** Routine surveys for larvae and adult mosquitoes are carried out once a month at nine sentinel sites (one per district). Reports are sent to local health offices for appropriate action.
- **Funding:** The PRP is funded totally by the Government of Mauritius, of which the annual cost per capita is US$2.06 (2008 US$) or 0.83% of total public health expenditure [5].
- **Other malaria control interventions:**
  In addition to LSM, the cornerstones of the Mauritius PRP are:
  - Epidemiological and entomological surveillance
  - Good case diagnosis and management, free health care (prophylaxis and treatment)
  - Vector control
    - DDT spraying at ports of entry (although this is currently being phased out)
    - Aircraft disinsection
  - Monitoring & evaluation
  - Health education

  Long lasting impregnated nets (LLINs) and mass drug administration (MDA) are not currently used in Mauritius. LLINs are provided only to known malaria inpatients.

Figure 2. *Larviciding*

Impact

No autochthonous cases of malaria have been reported in Mauritius since 1997. It is difficult to assess the degree to which the success of the PRP can be attributed to LSM. Certainly, other factors may have contributed to the prevention of reintroduction of malaria into Mauritius. Specifically, LSM in Mauritius is a compliment to a robust surveillance system and a passenger screening program which reduce the risk of importation and indigenous transmission [5]. A recent review and analysis aimed to assess the impact of the passenger screening arm of the PRP and concluded that this program suppresses the risk of indigenous transmission of approximately 1.7%–7.5% each year [5]. To our knowledge, the impact of the LSM component of the PRP remains to be established.

Challenges

- Mauritius remains **receptive** to malaria since the vector *An. arabiensis* is still present.
- The **risk of re-introduction** of malaria persists due to:
  - The close proximity of Mauritius to malaria endemic countries.
  - The influx of tourists into the country.
  - The influx of migrant workers from endemic countries (particularly India, Pakistan, Bangladesh and Madagascar).
  - Mauritian nationals travelling to malaria endemic countries.
- Low immunity in the population to the malaria parasite.

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Larval Source Management (LSM) in urban India

The Indian National Malaria Eradication Programme (NMEP) was launched in 1958 using indoor residual spraying (IRS) for all roofed structures, except for those in urban areas where larval source management (LSM) was recommended [2]. While malaria incidence declined overall, urban malaria increased during the 1960s especially in the states of Tamil Nadu, Andhra Pradesh, Gujarat, Rajasthan and Maharashtra, partly due to the expansion of urban slums, population movement and lack of adequate waste water disposal. This was first recognised as a specific public health problem in 1969 by the Madhok Committee. The Urban Malaria Scheme (UMS) was sanctioned in 1971 and 23 towns were initially selected, before the Scheme was expanded to 131 towns in 19 states, the population of which was 112 million in 2009 [1]. The major objective of the UMS is to control malaria through good case management and LSM, since IRS is not accepted by the majority of the urban population [2]. The UMS targets the vectors of dengue, filariasis and Japanese Encephalitis in addition to malaria vectors. This document outlines the general structure of the UMS and its impact.

Background

- **Topography:** The Indian subcontinent (Fig. 1) is characterised by nearly every major type of landscape, from grass steppes and fertile flood plains to arid desert and mountains.
- **Climate**: There are six main climatic regions: alpine, sub-tropical humid, tropical wet-dry, tropical wet, semi-arid and arid. There are four seasons: winter (January-February), summer (March-May), monsoon (rainy) season (June-September) and post-monsoon season (October-December).

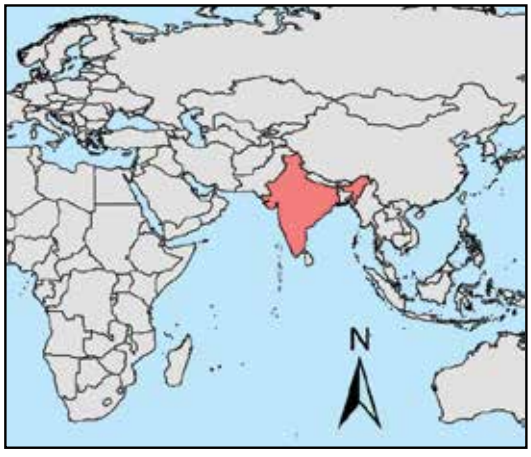

Figure 1. *India*

- **Primary and secondary vectors**: The major vectors in urban areas are *Anopheles stephensi* and *An. culicifacies.*
- **Main type of breeding sites:** *An. stephensi* breeds mainly in man-made wells and cisterns; *An. culicifacies* breeds in agricultural grassland typically found in peri-urban areas.
- **Malaria transmission:** Transmission is low and perennial with peaks in both *Plasmodium falciparum* (which accounts for around 50% of total cases [3]) and *P. vivax.*

The larval source management program

- **Structure of the control program:** 131 towns are included in the UMS. For a town to be included in the scheme the population must exceed 50,000, the annual parasite incidence must exceed 2 per 1000 people and civic bye-laws to prevent or eliminate domestic and peri-domestic breeding paces should be in place [2]. At town level, the UMS should be run by a Biologist and supervised by State and Central Health Authorities. Every target municipal area of each town is ideally divided into wards of 25.6 km^2 (10 $mile^2$), which are further divided into sectors of 2.56 km^2 (1 $mile^2$) [2]. Each ward should have one Inspector and one Insect Collector and each sector has one Supervisor Field Worker and up to two Field Workers (depending on the quality of the drainage system). One driver and vehicle is provided per 40 sectors [2].
- **Baseline mapping and data collection:** prior to LSM, it is recommended that geographical reconnaissance (GR) is conducted and maps showing all breeding sites prepared. All breeding sites are also numbered. Breeding places where mosquito larvicidal oil (MLO) cannot be applied (e.g. agricultural fields, ornamental tanks, coconut husk resting ponds, septic tanks) are marked for treatment with larvicides [2]. Baseline data on larval and adult densities should be collected

before larvicide treatment. Susceptibility tests for temephos and fenthion are conducted as part of baseline data collection using the standard WHO technique [2].

***Figure 2**. Inaccessible overhead storage tank in Chennai (photo courtesy of Dr Rajander Sharma)*

- **Larval source management activities:**

  LSM should be conducted year-round, since malaria transmission is perennial [2].

  Environmental management: Habitat manipulation and modification (e.g. intermittent irrigation) are recommended by the UMS for use at the discretion of individual towns. For example, states and towns are responsible for planning and executing minor engineering works to permanently remove breeding sites through drainage or filling for example [2]. Civic bye-laws exist in some locations (e.g. Municipal Corporation of Greater Mumbai, National Capital Territory of Delhi, Chandigarh, Bhopal, Agartala, Navi Mumbai Municipal Corporation, Thane and Goa) which stipulate that individuals must help eliminate domestic and peri-domestic breeding places. Building bye-laws are also implemented in some towns (e.g. Navi Mumbai Corporation) which require precautions to be taken to prevent conditions for vector breeding on the exterior of buildings and curing tanks to be kept larvae-free during construction and dismantled before the issuing of occupancy certificates [1].
  Biological control: Larvivorous fish such as *Gambusia affinis* and *Poecilia reticulata* may be used for biological control where chemical control is not feasible [4].

  Larviciding: Mosquito Larvicidal Oil (MLO), pyrrethrum extract, temephos, fenthion and *Bacillus thuringiensis israelensis* are recommended by the UMS for use at the discretion of individual towns [2]. The same spray teams are deployed for both MLO and larvicides [2].

- **Entomological surveillance:** It is recommended that entomological surveillance is conducted by an Insect Collector who is responsible for surveying one ward, which is divided into 12 sections each with two fixed catching stations. Larval susceptibility tests are carried out every six months [2].
  - **Larval surveillance:** It is recommended that the area around two fixed catching stations is checked for fourth instar larvae and pupae for 30 minutes per day by the Insect Collector, using 5-10 dips with a 90mm dipper. Sampling is conducted between 8am and 10am and morphological identification carried out in the afternoon [2]. Cross-checking is conducted by a Malaria Inspector and Insect Collector. Larval density is recorded in standard forms and reported monthly to the State Malariologist or State Programme Officer and the Director of the National Anti Malaria Programme (previously known as the NMEP) in Delhi [2].
  - **Adult mosquito surveillance:** Ideally, mosquito collections are conducted six days per week at four fixed and five randomly-selected catching stations between 6.30am and 8.30am. Mosquito resting places within houses are actively searched for 15 minutes and mosquitoes collected with a suction tube, which are transferred to a test-tube and morphologically identified on the same day. Mosquitoes are classified to the genus level (anophelines and culicines) and in addition numbers of *An. stephensi, An. culicifacies, Culex quinquefasciatus* and *Aedes aegypti* are recorded. Overall, each fixed catching station is visited once a week [2].

- **Funding:** Initially, the UMS was centrally sponsored by the Government of India. Since 1979-80, the costs of the UMS have been split equally between the central and state governments [2].

- **Other malaria control interventions:** Insecticide-treated nets are distributed for free to all age groups; IRS is used in rural areas however coverage remains low [3].

Impact

It is difficult to directly measure the impact of the UMS. Critics of the Scheme have highlighted that even now, the UMS operates only in 133 towns and cities and in many other urban areas, the municipality administrators are responsible for malaria control. In many smaller urban and industrial settings there is no malaria control system [5].

There are also difficulties in measuring the exact burden of malaria in India [6]. A retrospective study using UMS and health facility data indicated that the mean annual incidence of malaria in Ahmedabad city, 1991-1998, was 12.2 cases per 1000 population, which far exceeds the rate officially reported by the UMS (1.3 cases per 1000 population) [5]. Verbal autopsy data collected between 2001-2003 indicated that 205,000 deaths (95% CI 125,000-277,000) per year could be attributed to malaria in India [7], while WHO estimates indicated that only 15,000 deaths (95% CI 9,600-21 ,000) could be attributed to malaria in India in 2006 [8]. Similarly, Snow and colleagues used spatial estimates of the limits and intensity of malaria transmission and estimated the clinical malaria burden in India in 2007 to be 101.5 million clinical cases (95% CI 31.0-187.0 million) [9], also higher than WHO estimates [7].

Although there was a decline in malaria in the first 5 to 6 years after the introduction of the UMS, there has been a well-documented increase in malaria in parts of India in recent decades, for example in Kolkata [10, 11], Madras [12] and Mumbai [13]. In Kolkata, health facility data for 1984-1997 demonstrated an increase in mean annual incidence from around 40-60 cases per 1,000 during 1984-87 to 50-70 per 1,000 during 1994-97 [14]. Similarly, between 1992 and 1997, there was a resurgence of malaria in Mumbai, where the disease remains a significant public health problem [13]. This has been partly attributed to the increase in construction

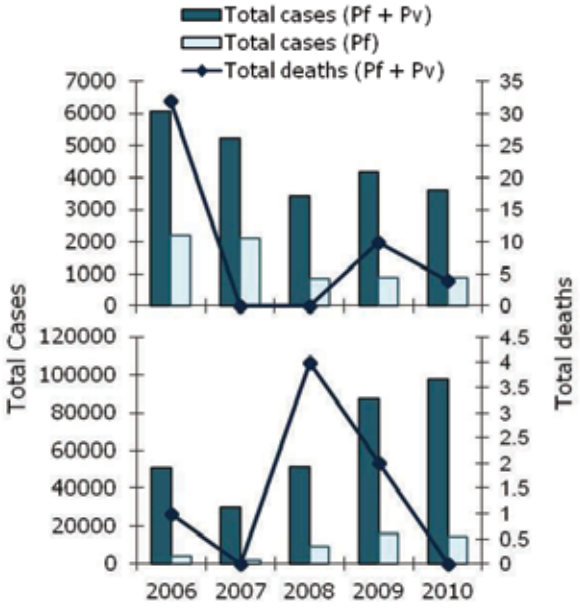

Figure 3. *Total reported cases and deaths in Ahmedabad (top) and Kolkata (bottom) between 2006 and 2010 [1].*

activities after economic changes in 1991 and the effect of local politics on the administration of the Municipal Corporation of Greater Bombay [13], together with chloroquine resistance [15].

In general, political, social, economic and administrative problems have hampered the implementation of the UMS in India. The resurgence in malaria may have also been associated with rapid urban growth and improvements in drainage and sanitation systems lagging behind the UMS. Environmental measures have therefore not been implemented well, and this, combined with the temporary effect of biological control and larviciding, has limited the success of the UMS in cities such as Madras [12].

Today, urban malaria remains a public health problem in India, especially in the cities of Mumbai, Chennai, Kolkata (Fig. 3) & Mangalore [16]. However UMS data indicates that malaria has declined in some towns such as Ahmedabad and there is evidence that in other towns LSM may have contributed to a decline in malaria [17, 18]. For example, in the area around Bharat Heavy Electricals Ltd in the township of Hardwar, expanded polystyrene beads, larviciding and larvivorous fish were associated with a reduction

in the total number of confirmed reported malaria cases from 3049 in 1985 to 190 in 1995 [19].

Challenges

Malaria mortality has increased in Mumbai, Chennai, Kolkata and Mangalore among other cities, partly due to:

- Inaccessibility of many overhead storage tanks (e.g. 30% of tanks are inaccessible in Chennai) [1].
- Continuous population growth in urban areas increases pressure on water systems, increasing vector breeding [1].
- Expansion of peri-urban areas with poor infrastructure has increased the number of *An. culicifacies* breeding sites.
- Vertical expansion of cities creates new breeding sites since fire regulations stipulate that both ground and roof water storage tanks must be added to buildings [1].
- Water being stored in artificial containers because the water supply is intermittent [1].
- Inadequate health infrastructure and in particular a lack of man-power [1].
- Immigration from endemic rural areas to urban areas (e.g. Kolkata and Ahmedabad [1].
- Incorrect implementation of recommendations of the UMS [1, 2].

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Annex

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