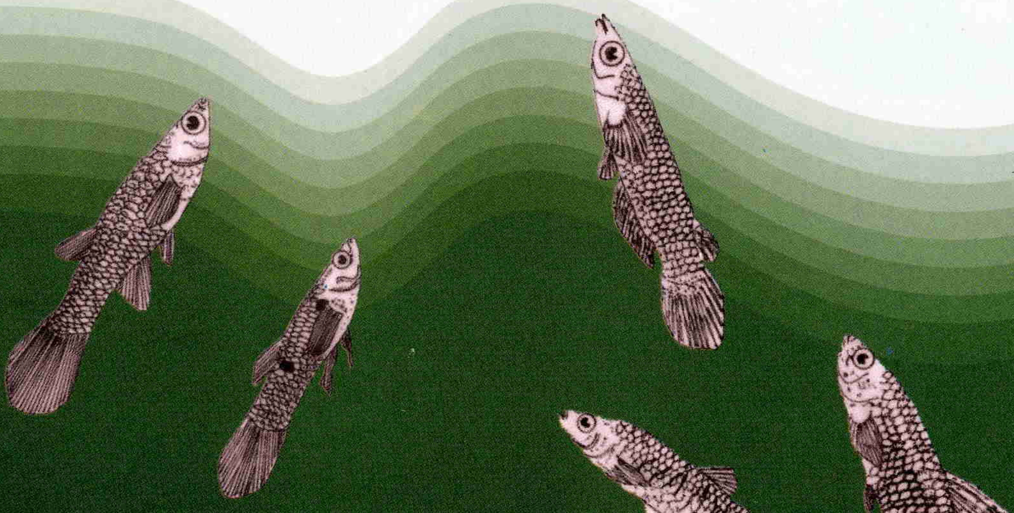
WHO-EM/MAL/289/E/G
Distribution: General

Use of fish for mosquito control



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Regional Office for the Eastern Mediterranean

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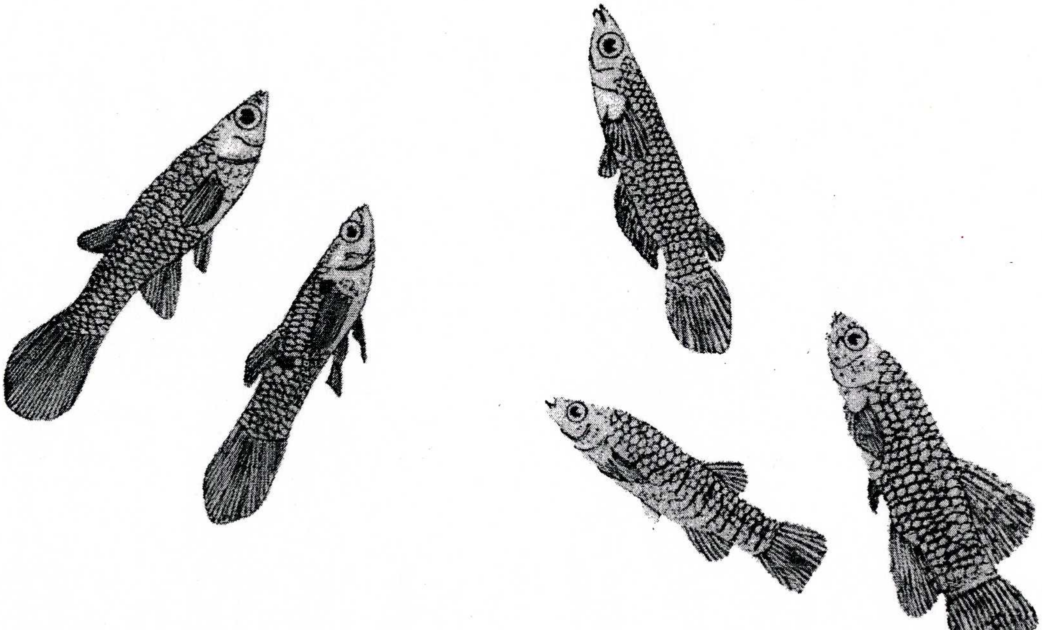

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Contents

Preface

In countries of WHO's Eastern Mediterranean Region, vector-borne diseases contribute significantly to the burden of communicable diseases. The WHO Regional Office for the Eastern Mediterranean is currently promoting the use of various vector control strategies in a cost-effective manner to target more than one vector-borne disease, and strategies that rely less on the use of insecticides are particularly appealing. One such strategy is the use of certain species of fish that feed on mosquito larvae (vectors of malaria, bancroftian filariasis and arboviral infections). This strategy targets the larvae rather than the adult mosquitoes and is potentially safer for humans as it does not involve use of insecticides. However the method is only suitable for use where mosquito vector breeding is well defined and where transmission is seasonal. The use of larvivorous fish has been introduced in several countries of the Region, and its potential and methods of implementation have been tested in two countries.

This document has been developed as a guide to countries of the Region as they embark on the implementation of integrated vector management for the control of mosquito-borne diseases. It describes regional experience to date with use of larvivorous fish and the role of this intervention in mosquito-borne disease control; regional and global distribution of indigenous and some exotic fish species with potential for larval feeding and/or eliminating aquatic weeds; methods/guidelines of establishing fish nurseries; monitoring and evaluation of impact; and community and intersectoral participation in developing larvivorous/herbivorous fish resources and use of fish.

The document is intended for use by personnel engaged in anti-malaria or anti-mosquito services at the national, provincial, district, primary health care centre and village levels; in nongovernmental organizations at the community level; and in non-health sectors, such as industry, armed forces, institutions, railways, ports and airports. The document can also be used for training and reference purposes for national managers of vector control programmes in the Region.

Acknowledgements

The Regional Office would like to acknowledge the work of all those who contributed to developing this manual, both within and outside the WHO Regional Office for the Eastern Mediterranean, and especially that of Dr Rajpal S. Yadav, Malaria Research Centre (Indian Council of Medical Research).

Abbreviations

An.	:	*Anopheles*
°C	:	degree Celsius
cm	:	centimetre
DDT	:	dichloro-diphenyl-trichloroethane
ha	:	hectare
kg	:	kilogram
km	:	kilometre
$KMnO_4$	:	potassium permanganate
l	:	litre
m	:	metre
mg	:	milligram
ml	:	millilitre
mm	:	millimetre
NaCl	:	sodium chloride
pH	:	hydrogen-ion concentration
ppm	:	parts per million
MOC	:	*mahua* oil cake
sp.	:	species (singular)
spp.	:	species (plural)
s.l.	:	sensu lato
s.str.	:	sensu stricto
w.d.p.		water dispersible powder

1. Introduction

Background

During the pre-DDT era, control of mosquitoes and mosquito vectors of disease was undertaken mainly by environmental management, pyrethrum space spraying, use of Paris green, oiling with petrol products and larvivorous fish. Recognizing the high larvivorous potential of *Gambusia affinis*, this fish species was purposely introduced from its native Texas (southern USA) to the Hawaiian Islands in 1905. In 1921 it was introduced in Spain, then from there into Italy during the 1920s and later to 60 other countries [1]. Beginning in 1908, another larvivorous fish, *Poecilia reticulata* (guppy), a native of South America, was introduced for malaria control into British India and many other countries. The introduction of the use of DDT in indoor residual spraying for malaria control around the mid-1940s led to the gradual decline in the use of environmental management and biological control methods except in a few programmes in Russia.

Importance of biological control of vectors and WHO initiatives

Following the resurgence of malaria in the early 1970s in some countries, reports of the development of insecticide resistance in malaria vectors and increased fear of environmental pollution with insecticides, interest in developing environment-friendly methods such as the use of biological control agents was renewed. A major boost in this direction was given by a travelling seminar in 1979 on the use of larvivorous fish for mosquito control under the auspices of WHO. This was followed by further consultations among members of the WHO expert committee on vector biology and control and experts of the WHO informal consultative meeting on the role of larvivorous fish, either alone or in an integrated approach, for control of mosquito-borne diseases [2,3]. Global experience has shown that in many situations fish are effective in controlling mosquito-borne disease either alone or as part of an integrated control approach. Two major annotated bibliographies have been compiled on the use of fish in mosquito control [1,4].

Promotion of the use of fish in vector control in the Region

Malaria is the main mosquito-borne disease of public health importance in the countries of the Eastern Mediterranean Region of WHO (hereinafter referred to as 'the Region'). Currently, rough estimates are that 15 million malaria cases appear annually, mainly from Afghanistan, Djibouti, Somalia, Sudan and Yemen. Dengue, Rift Valley fever, severe outbreaks of yellow fever, other arboviral infections and filariasis are also reported from some countries in the Region. The main methods of control of the vectors of these diseases are indoor residual spraying of insecticides, larviciding and the use of insecticide-treated mosquito nets. Under WHO's Roll Back Malaria initiative, the Regional Office for the Eastern Mediterranean is currently promoting the use of integrated methods of vector control in the Region. Use of fish is an important component of this strategy. In many countries of the Region, local and exotic species of fish are being used in larval control, details of which are presented in Chapter 3. In 2001, the Regional Office obtained the services of a consultant to promote these efforts. Other regions of WHO (e.g. the European Region and the South-East Asia Region) are also promoting the use of larvivorous fish for mosquito control.

Rationale for larval control using fish

In specific situations where mosquito breeding habitats are well defined and water conditions are suitable, or where chemical larviciding is not feasible, indigenous or exotic fish with a known larvivorous potential can be used for larval control. Although larvivorous fish have been used extensively based on the empirical knowledge, certain scientifically designed trials have proved the operational efficiency of their use in malaria control. Use of *Oreochromis spilurus spilurus* (tilapia fish) was evaluated by the UNDP/WHO/World Bank Special Programme for Research and Training in Tropical Diseases (TDR) in northern Somalia during 1980–82 for its control of rural malaria transmitted by *Anopheles arabiensis* breeding in the man-made, rainwater filled, cement-lined reservoirs called "birkets" [5]. Another randomized trial reported the efficiency of *Aphanius dispar* in controlling the larvae of *An. arabiensis* in the port city of Assab in Ethiopia [6]. In Hyderabad city, India, an operational release of *Gambusia affinis holbrooki* in 1967 controlled the breeding of *An. stephensi* in hundreds of wells in about 2 years [7].

Experience has shown that biological control using fish is best achieved as part of an integrated vector control strategy. While larval control by fish, like chemical larviciding, will reduce vector densities, a nearly perfect larval control is required to significantly reduce the risk of malaria transmission (or vectorial capacity) in a given area. A nearly complete larval control is possible in well defined situations (semi-arid areas, oases, urban areas, etc.) and would require, among other things, a thorough knowledge of the vector ecology, geographical reconnaissance of larval habitats in targeted areas and a significant degree of skill in breeding, transportation and use of fish. Certain species have food value, while others help clear weeds in water bodies, improving the environment. Indigenous fish are generally preferred over the exotic species since they are well adapted to the local environment. Caution should be exercised during the introduction of exotic species in new areas (see Chapter 5).

Gerberich and Laird reviewed the issue of the cost of using larvivorous fish [8]. Studies in California reported that the use of *Gambusia affinis* alone or in combination with *Oreochromis* (*Tilapia*) *zillii* was economical, especially in the long run, compared with repeated chemical treatments of the same site [9,10]. In Afghanistan, the total operational cost of introducing *Gambusia* for malaria control was US$ 0.02 per capita (1972), compared with US$ 0.50 per capita (1971) using DDT (75% w.d.p.) indoor residual spraying [2]. Fish can be used cost-effectively, especially when there is also community participation, such as in the maintenance of fish stocks and hatcheries, removal of weeds from water bodies, distribution of fish, replenishment of fish in mosquito breeding habitats and the use of larvivorous fish with food value.

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2. Zoogeographical ecosystems, malaria vectors and their ecology in the Region

Three zoogeographical regions meet in the Eastern Mediterranean Region: the Afrotropical region, the Oriental region and the Palaearctic region.

Among the countries of the Region, Djibouti, Somalia, Sudan and western/south-western parts of Yemen and Saudi Arabia lie in the Afrotropical region, which is characterized by high malaria endemicity maintained by a very efficient malaria vector, *Anopheles arabiensis. An. arabiensis* is the principal malaria vector in the drier savannah areas from Sudan to Somalia, Yemen and Saudi Arabia. It often bites humans and rests indoors, but also feeds on cattle and rests outdoors. Other malaria vector species of the region are *An. pharoensis*, whose distribution extends up to Egypt in the north, and *An. gambiae* s.str., the presence of which is limited to southern Sudan.

The Oriental region, encompassing Afghanistan, south-eastern Iran, Oman and Pakistan,[1] is hypo-endemic to meso-endemic for malaria. The main malaria vectors in this region are *An. culicifacies*, *An. fluviatilis* and *An. stephensi*. Most of these species, however, are groups of sibling species and some member species of each of these groups can be malaria vectors and others not.

In the Palaearctic region, which encompasses the remaining countries of the Region, the incidence of malaria is either low or there is no malaria transmission at present. However, the region has the potential to become meso-endemic and even hyper-endemic. The main vector species are *An. labranchiae*, *An. sergenti*, *An. sacharovi* and *An. superpictus.* Other important vectors in some countries of the Region are *An. pulcherrimus*, *An. maculipennis s*.str. in northern Iran, *An. claviger* in the Levant and probably *An. hyrcanus* species complex in Afghanistan.

The malaria vectors in the countries of the Region are given in Table 1 [11, 12]. Main breeding habitats of the vectors of malaria in countries of the Region are shown in Table 2.

[1] South-eastern Iran and Oman are part of the Oriental region, although some representatives of the Oriental fauna are found in all member countries of the Gulf Cooperation Council and in Yemen.

Table 1. **Malaria vectors in the countries of the Eastern Mediterranean Region**

Country	Vectors of malaria: ***Anopheles***
Afghanistan*	*culicifacies, stephensi, pulcherrimus, superpictus, hyrcanus complex*
Bahrain	*stephensi*
Cyprus	*sacharovi, claviger, superpictus*
Djibouti	*arabiensis*
Egypt	*pharoensis, sergenti*
Iran, Islamic Republic of	*stephensi, culicifacies, dthali, maculipennis* s.str, *sacharovi, superpictus, fluviatilis, pulcherrimus*
Iraq	*stephensi, sacharovi, superpictus, pulcherrimus*
Jordan	*sergenti, superpictus, sacharovi*
Kuwait	*stephensi*
Lebanon	*sacharovi, superpictus, claviger*
Libyan Arab Jamahiriya	*sergenti*
Morocco	*labranchiae, sergenti*
Oman	*culicifacies, stephensi, fluviatilis, sergenti*
Pakistan	*culicifacies, stephensi, fluviatilis, superpictus*
Palestine	*sergenti, superpictus, claviger*
Qatar	*stephensi*
Saudi Arabia	*arabiensis, sergenti, stephensi*
Somalia*	*arabiensis, funestus*
Sudan*	*arabiensis, funestus, pharoensis, gambiae* s.str., *nili, coustani*
Syrian Arab Republic	*sacharovi, superpictus, claviger, multicolor*
Tunisia	*labranchiae, sergenti*
United Arab Emirates	*culicifacies, stephensi*
Yemen*	*arabiensis, sergenti, coustani, culicifacies (adenensis)*

*These countries together contribute about 98% of all reported cases of malaria in the Region

Table 2. **Main breeding habitats of the principal malaria vectors in countries of the Eastern Mediterranean Region**

Vector	Breeding habitat
An. arabiensis *An. gambiae*	shallow standing or moving water with algae in streams in valleys, moderately polluted household wastewaters, tap-water puddles/grassy channels, rainwater pools, irrigation channels, cisterns, dykes in canals
An. claviger	ponds, marshes, springs, wells, cisterns, rock pools
An. culicifacies	small pools, impoundments, shallow sunlit wells, river-bed pools, rain puddles
An. fluviatilis	sunlit hill streams, slow running waters, channels with grassy margins, pools in riverbeds
An. funestus	permanent waters especially with vegetation such as swamps, marshes, ditches, edges of streams, rivers
An. labranchiae	sunlit ponds overgrown with vegetation, rice fields, residual pools, blind ends of streams, brackish waters in coastal marshes
An. pharoensis	shaded, clear, stagnant and shallow waters having a thick growth of vegetation, drains and irrigation channels with stagnant water, seepages, pools and borrow pits with vertical vegetation, rice fields, marshes
An. pulcherrimus	borrow pits with vegetation, rice fields, shallow lakes
An. sacharovi	small water collections having aquatic vegetation, water-filled pits along river banks, neglected ponds, wide canals, blind ends of streams, and rain puddles
An. sergenti	rice fields, borrow pits, ditches, seepages, relatively high salinity content, clean, shallow and slow flowing streams (sunlit or partially shaded), agricultural drains, irrigation channels
An. stephensi	man-made water storage containers, overhead tanks, cemented reservoirs, cisterns, wells, ornamental or fountain pools, curing water tanks at construction sites, grassy pools especially alongside rivers
An. superpictus	breeds in pools with high calcium content, shallow water left by torrents over rocky streams, pools in rivers, muddy hill streams and under pebbles

3. Potential larvivorous fish in the Region

Characteristics of a potential larvivorous fish

On the basis of their food preferences, fish are generally classified as:

- **herbivorous**: feeding mainly on substances of plant origin,
- **carnivorous**: feeding mainly on substances of animal origin, or
- **omnivorous**: feeding on substances of plant and animal origin.

Fish can be further classified as:

- **planktonivorous**: feeding mainly on phyto-planktons and zoo-planktons, or
- **larvivorous**: feeding mainly on insect larvae and pupae.

For anopheline control, a potential larvivorous fish should possess the following characteristics:

- have a high preference for mosquito immatures among a wide variety of food, e.g. plankton available in the natural habitats;
- be a surface feeder, since anopheline larvae stand on the surface strata of aquatic habitats;
- have terminal or superior mouth with teeth and swim above the surface stratum of aquatic habitats;
- be small in size, more or less fusiform in shape, agile and swift in movement so that it can navigate in shallow and/or weed-infested waters and escape from the predators;
- have high fecundity (breeding potential) and ability to breed throughout the year under natural conditions and in confined waters; and
- be hardy to withstand transportation and stressful environmental conditions in terms of temperature, pollutants and turbidity

Preliminary assessment of larvivorous efficacy of a fish species

When the larvivorous potential of a new fish species is not known, there are several ways to make a preliminary assessment as to whether a fish predominantly eats mosquito larvae:

1. Dissect out the contents of the intestine of a fish collected from its natural habitat and observe under a binocular dissecting microscope the presence of remains of mosquito larvae (head, body cuticula of digested larvae and pupae, siphon of *Culex* larva, pecten of *Anopheles* larva) and *Anopheles* eggs.
2. Put a fish collected from its natural habitat into a container, the next day concentrate the sediments in a conic recipient, collect with a pipette the faecal matter, put it on a microscope slide, absorb the excess of water on a blotting paper and add a drop of glycerol, place a cover slip and observe for the remains of larvae/eggs under a dissecting microscope/hand lens.
3. Carry out laboratory experiments to determine the number of third/fourth instars a fish eats in 24 hours. In a glass container with 2 litres of water, add five fish and a known number of third/fourth instars (this would vary according to the size and type of the fish, so determine by initial experiment). Run five replicates. Observe consumption of the larvae periodically and count the number of larvae remaining in the container after 24 hours. The experiment can be designed in a number of ways: observe daily feeding rate for different sizes/weights of the fish in the presence/absence of other natural foods such as zoo-/phyto-planktons. Calculation of the consumption of the number of larvae per unit of weight is a standard measure of the larvivorous potential of a fish in laboratory experiments [13,14].

Small-scale field trials are designed to measure the impact of the fish on larval densities in natural habitats of the mosquito/vector species. Select about 100 small larval habitats or isolated plots and after determination of the baseline larval densities (say for about 4 weeks) randomize them into two groups, one receiving intervention with fish and the other serving as control. Introduce fish (number to be decided according to the size of the habitat; application rate may be five fish/m^2 or more) and measure the impact of the fish on larval densities after day 2 and thereafter weekly/every tenth day during the main malaria season. Relative reduction in larval densities may then be calculated.

Species of fish that can be used for mosquito control

Fish species known to have larvivorous and herbivorous potential in different regions of the world and their bionomics are shown in Table 3. As indicated, the most promising larvivorous fish belong to the families Poeciliidae, Cyprinidae, Cyprinodontidae and Chichlidae.

Fish species with proven larvivorous potential in the Eastern Mediterranean Region are:

Indigenous species

Aphanius dispar
Aplocheilus panchax
Aplocheilus blockii
Aplocheilus lineatus
Nothobranchius patrizii
Nothobranchius cyaneus
Nothobranchius guentheri
Nothobranchius microlepis
Oreochromis spilurus spilurus
Oreochromis niloticus
Oreochromis zillii
Puntius ticto
Puntius sophore
Rasbora daniconius

Exotic/Introduced species

Gambusia affinis
Gambusia holbrooki
Poecilia reticulata

The geographical distribution of the known potential indigenous and exotic larvivorous fish in the countries of the Eastern Mediterranean Region is indicated in Figure 1 and they are further described in Annex 1. Known phytophagous and malacophagous fish are also discussed in Annex 1.

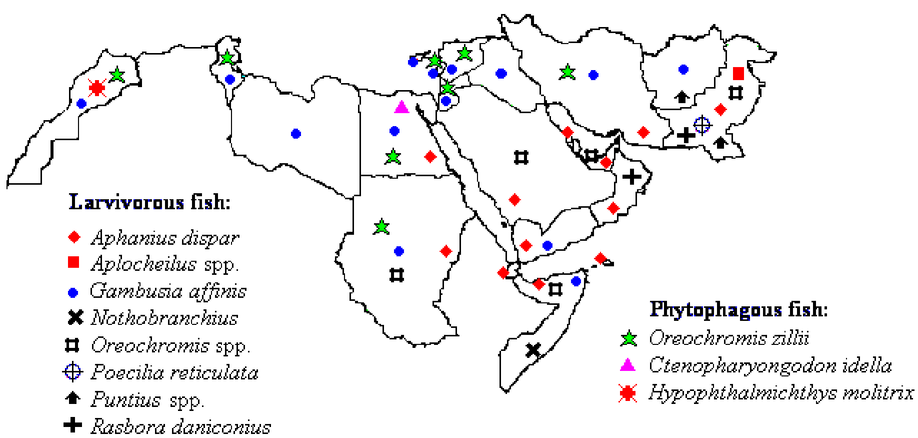

Figure 1. **Distribution of known larvivorous and phytophagous fish in the countries of the Eastern Mediterranean Region**

Table 3. **Bionomics and tolerances of fish with known larvivorous and herbivorous potential**

Bionomics and tolerances	**Zones/countries**		
	Mediterranean and Near East (including Afghanistan and Islamic Republic of Iran)	**Morocco, Spain**	**Pakistan, South-East Asia**
Bionomics			
Family	Cyprinodontidae	Cyprinodontidae	Cyprinodontidae
Genus, number of species	*Aphanius*, 12	*Valencia*, 1	*Aplocheilus*, 4
Well-investigated species	*dispar*, *sophiae*, *mento*	*hispanica*	*lineatus, dayi, blocki, panchax*
Length (cm)	5–10	8–10	5–12
Dimorphism of sexes	Intermediate	Little	Intermediate
Type of spawning	On plants and substratum	On plants and substratum	On plants and substratum
Food	Omnivorous	Omnivorous	Carnivorous
Incubation period (days)	7–10	12–14	14
Resistance of eggs to desiccation	Not resistant	Not resistant	Not resistant
Size of fry	Medium	Big	Big
Rate of growth	Rapid	Rapid	Rapid
Position of mouth	Superior	Superior	Superior
Breeding period	Year round (depends on temperature)	Year round (depends on temperature)	Year round (depends on temperature)
Habitat	Streams, lakes, pools, ponds	Streams, lakes, pools, ponds	Lentic
Water chemistry tolerance			
PH	6–8	6–8	6–8
Degree of hardness	Hard	Hard	Moderate
Salinity	Tolerant to sea water	Tolerant to sea water	Not very tolerant
Organic pollution	Resistant	Resistant	Resistant

Table 3. **Bionomics and tolerances of fish with known larvivorous and herbivorous potential (cont.)**

Bionomics and tolerances	**Zones/countries**		
	Mediterranean and Near East (including Afghanistan and Islamic Republic of Iran)	**Morocco, Spain**	**Pakistan, South-East Asia**
Water temperature tolerance			
Max. range (°C)	8–40	10–25	18–40
Min. (for breeding) (°C)	16–26	16–18	23–28
Remarks	Very adaptable, hardy, voracious feeders	Tolerate cooler waters, voracious feeders	Surface feeder
	East Africa (including Somalia and Sudan)	**East Africa**	**East Africa (including Somalia and Sudan)**
Bionomics			
Family	Cyprinodontidae	Cyprinodontidae	Cichlidae
Genus, number of species	*Nothobranchius*, 20–25	*Pachypanchax*, 2	*Oreochromis*, >20
Well-investigated species	*guentheri, orthonotus, patrizii, korthausae*	*Playfairi, pomalonotus*	*spilurus spilurus, nilotica, mossambica, zillii*
Length (cm)	5–8	6–10	16–20
Dimorphism of sexes	Strong	Little	Little
Type of spawning	In substratum	On plants	On substratum, some carry eggs in the mouth
Food	Carnivorous	Carnivorous	Omnivorous, *O. zillii* is herbivorous
Incubation period (days)	20–365 (eggs require drying)	14	4–6
Resistance of eggs to desiccation	Resistant	Not resistant	Not resistant
Size of fry	Small	Medium	Large
Rate of growth	Extremely rapid	Extremely rapid	Rapid
Position of mouth	Superior	Superior	Terminal

 Use of fish for mosquito control

Table 3. **Bionomics and tolerances of fish with known larvivorous and herbivorous potential (cont.)**

Bionomics and tolerances	**Zones/countries**		
	East Africa (including Somalia and Sudan)	**East Africa**	**East Africa (including Somalia and Sudan)**
Bionomics			
Breeding period	Year round	Year round	Year round Parental care found
Habitat	Temporary ponds	Streams, pools, ponds	Streams, ponds
Water chemistry tolerance			
PH	6–8	6–8	6–8
Degree of hardness	Soft and hard	Soft and hard	Variable tolerance
Salinity	Tolerant	Very tolerant	Variable tolerance
Organic pollution	Very resistant	Resistant	Resistant
Water temperature tolerance			
Max. ranges (˚C)	16–30	18–35	10–40
Min. (for breeding) (˚C)	20–25	23–28	18–28
Remarks	“Annual”, Voracious feeders	Voracious feeders	Can be a food fish
	West Africa	**West Africa**	**West Africa**
Bionomics			
Family	Cyprinodontidae	Cyprinodontidae	Cyprinodontidae
Genus, number of species	*Epiplatys*, 30	*Aplocheilichthys*, 50	*Procatopus*, 10
Well-investigated species	*fasciolatus, dageti, chaperi*	none	*gracilia*
Length (cm)	3–10	2–6	5–8
Dimorphism of sexes	Strong	Little	Very little
Type of spawning	On plants	On plants	On plants
Food	Carnivorous	Carnivorous	Carnivorous

Table 3. **Bionomics and tolerances of fish with known larvivorous and herbivorous potential (cont.)**

Bionomics and tolerances	Zones/countries		
	West Africa	**West Africa**	**West Africa**
Bionomics			
Incubation period (days)	14	10–18	10–14
Resistance of eggs to desiccation	Not resistant	Not resistant	Not resistant
Size of fry	Small	Small	Small
Rate of growth	Rapid	Rapid	Rapid
Position of mouth	Superior	Superior	Superior
Breeding period	Year round	Year round	Year round
Habitat	Slow streams, pools, ponds	Slow streams, pools, ponds	Rapid streams
Water chemistry tolerance			
PH	6–8	5–7.5	6–8
Degree of hardness	Soft and hard	Soft	Soft
Salinity	Slightly tolerant	Slightly tolerant	Slightly tolerant
Organic pollution	Resistant	Not resistant	Not resistant
Water temperature tolerance			
Max. ranges (°C)	15–35	16–30	16–30
Min. (for breeding) (°C)	20–25	20–25	20–25
Remarks	Surface feeders	Surface feeders	Surface feeders
Family	Cyprinodontidae	Cyprinodontidae	Anabantidae
Genus, number of species	*Aphyosemion*, 60	*Roloffia*, 20	*Macropodus, Betta, Colisa, Trichogaster, Trichopsis*
Well-investigated species	*calliurum, gardneri, sjostedti*	*occidentalis, geryi*	Many species in each genus
Length (cm)	5–12	5–10	3–15

Table 3. **Bionomics and tolerances of fish with known larvivorous and herbivorous potential (cont.)**

Bionomics and tolerances	**Zones/countries**		
	West Africa	**West Africa**	**South-East Asia**
Bionomics			
Dimorphism of sexes	Strong	Strong	Strong
Type of spawning	(1) On plants (2) On substratum	(1) On plants (2) On substratum	Nests on water surface (bubbles usually made by male)
Food	Carnivorous	Carnivorous	Carnivorous
Incubation period (days)	(1) 14 (2) 30-120	(1) 14 (2) up to 270	2–3
Resistance of eggs to desiccation	Partially resistant	(1) Partially resistant (2) resistant	Not resistant
Size of fry	Small or medium	Small or medium	Small
Rate of growth	Rapid	Rapid	Slow
Position of mouth	Superior	Superior	Terminal
Breeding period	Year round	Year round	Year round
Habitat	Slow streams, pools, ponds	Slow streams, pools, ponds	Slow warm reservoirs and slow streams
Water chemistry tolerance			
pH	5.5–7.5	5.5–7.5	6–9
Degree of hardness	Soft	Soft	Soft and hard water
Salinity	Some species slightly tolerant	Some species slightly tolerant	Slightly tolerant
Organic pollution	Resistant	Resistant	Very resistant
Water temperature tolerance			
Max. ranges (°C)	15–35	15–35	20–40
Min. (for breeding) (°C)	20–25	20–25	23–30
Remarks	Males may be territorial, surface feeders	Males may be territorial, surface feeders	Air breathing, easily transported

Table 3. **Bionomics and tolerances of fish with known larvivorous and herbivorous potential (cont.)**

Bionomics and tolerances	**Zones/countries**		
	South-East Asia	**South-East Asia**	**South-East Asia**
Bionomics			
Family	Cyprinidae	Hemirhamphidae	Cyprinodontidae
Genus, number of species	*Rasbora, Puntius, Chela, Danio, Brachydanio, Esomus, Osteobrama*, 1–3	*Hemirhamphus (Dermatogenys)*, 13	*Oryzias*, 10
Well-investigated species	Many species in each genus	None	*melastigma, javanicus, latipes*
Length (cm)	2–10	4–10	3–4
Dimorphism of sexes	Almost none	Little	Very little
Type of spawning	On substratum	Viviparous	On plants, eggs in clusters
Food	Omnivorous	Carnivorous	Carnivorous
Incubation period (days)	1–4	NA (no eggs laid)	3–5
Resistance of eggs to desiccation	Not resistant	NA (no eggs laid)	Not resistant
Size of fry	Small or of medium size	Big	Very small
Rate of growth	Slow	Rapid	Slow
Position of mouth	Terminal	Superior	Superior
Breeding period	Rainy season	Year round	Year round (temperature dependent)
Habitat	Streams, stagnant water, rivers, lakes, ponds	Lagoons and fresh reservoirs	Primarily still water
Water chemistry tolerance			
pH	6–8	7–9	7–9
Degree of hardness	Soft	Very hard	Hard
Salinity	Not tolerant	Tolerant to sea water	Tolerant to salinity
Organic pollution	Some can withstand, but generally poor	Resistant	Resistant

Table 3. **Bionomics and tolerances of fish with known larvivorous and herbivorous potential (cont.)**

Bionomics and tolerances	**Zones/countries**		
	South-East Asia	**South-East Asia**	**South-East Asia**
Water temperature tolerance			
Max. ranges (°C)	18–40	18–40	18–40; 1–40 (*latipes*)
Min. (for breeding) (°C)	23–28	23–28	23–28; 18–22 (*latipes*)
Remarks	Surface and shoaling feeders	Surface feeders	Surface feeders
	Latin America	**Latin America**	**Latin America**
Bionomics			
Family	Cyprinodontidae	Cyprinodontidae	Cyprinodontidae
Genus, number of species	*Rivulus*, 60	*Fundulus*, 31	*Cyprinodon*, 10
Well-investigated species	*cylindraceus, marmoratus*	*grandis, majalis*	*variegatus*
Length (cm)	5–10	6–15	4–8
Dimorphism of sexes	Strong	Little	Intermediate
Type of spawning	On plants	On plants	On substratum
Food	Carnivorous	Carnivorous	Omnivorous
Incubation period (days)	14	10	7–14
Resistance of eggs to desiccation	Not resistant	Not resistant	Not resistant
Size of fry	Big	Big	Small
Rate of growth	Rapid	Rapid	Rapid
Position of mouth	Superior	Superior	Superior
Breeding period	Year round	Year round	Year round
Habitat	Streams, pools, ponds, swamps	Streams, pools, ponds, swamps	Lagoons, desert pools, brackish water

Table 3. **Bionomics and tolerances of fish with known larvivorous and herbivorous potential (cont.)**

Bionomics and tolerances		**Zones/countries**	
	Latin America	**Latin America**	**Latin America**
Water chemistry tolerance			
pH	6–8	6–8	8–8.5
Degree of hardness	Soft and hard	Hard	Very hard
Water chemistry tolerance			
Salinity	Very tolerant	Very tolerant	Tolerant to sea water
Organic pollution	Partially resistant	Partially resistant	Resistant
Water temperature tolerance			
Max. ranges (˚C)	20–40	18–35	5–40
Min. (for breeding) (˚C)	25–32	20--25	20–25
Remarks	Active surface feeders	Active surface feeders	Very vigorous
	Latin America	**Latin America**	**Latin America**
Bionomics			
Family	Cyprinodontidae	Goodeidae	Poeciliidae
Genus, number of species	*Cynolebias*, 20	*Xenotoca, Goodea, Neotoca*, 7	*Gambusia, Poecilia, Girardinus, Xiphophorus*, 103
Well-investigated species	*belottii, adloffi, elegans*	*X. eiseni*	Many species in each genus
Length (cm)	4–12	6–14	2–8
Dimorphism of sexes	Strong	Marked	Males with gonopodium (anal fin in shape of tube)
Type of spawning	In substratum	Viviparous	Viviparous
Food	Carnivorous	Omnivorous	Omnivorous
Incubation period (days)	30–150 (eggs require drying)	NA (no eggs laid)	NA (no eggs laid)
Resistance of eggs to desiccation	Resistant	NA (no eggs laid)	NA (no eggs laid)

Table 3. **Bionomics and tolerances of fish with known larvivorous and herbivorous potential (cont.)**

Bionomics and tolerances	**Zones/countries**		
	Latin America	**Latin America**	**Latin America**
Bionomics			
Size of fry	Big	Very big	Big
Rate of growth	Very rapid	Very rapid	Very rapid
Position of mouth	Terminal	Terminal	Superior
Breeding period	Year round	Production of fry after about 50 days (*X. eiseni*) of gestation	Year round, production of fry after about 4 weeks of gestation
Habitat	Temporary pools	Streams, ponds, lagoons	Streams, ponds
Water chemistry tolerance			
pH	6–8	6–8	6–8
Degree of hardness	Soft and hard	Hard	Hard
Salinity	Slightly tolerant	Very tolerant	Tolerant
Organic pollution	Resistant	Resistant	Resistant
Water temperature tolerance			
Max. ranges (°C)	5–40	5–40	10–35
Min. (for breeding) (°C)	18–25	20–25	20–25
Remarks	Very vigorous	Very active feeders	*Poecilia* and *Gambusia* well known larvivorous

4. Review of past experiences and current use of larvivorous fish in the Region

Among the environment-friendly anti-larval methods, biological control is emerging as a major component of vector control. Larvivorous fish are used to control malaria in many countries of the Region, although there is a wide variance in knowledge of the relevant operational and technical aspects. A brief review by country of the experiences and current uses of larvivorous fish is given below.

Afghanistan

Gambusia affinis, the most widely used species in malaria control programmes worldwide, has been used in the Kunduz valley in Afghanistan. Rafatjah and Arata [15] reported that *G. affinis,* when introduced at a rate of 4–6 fish/m^2 water surface in rice fields, reduced the anopheline larval densities and vector biting rates sharply.

Thirty years ago *Gambusia affinis* were reared in the cities of Kunduz, Mazar, Faryab, Kabul, Lashkarga and Jalalabad in man-made rearing ponds established around perennial natural springs and maintained by teams of antimalaria workers headed by a supervisor in each region of the country (K.S. Mustafa, personal communication, 2001). A major fish breeding programme was implemented during 1971–1979. The fish were collected and transported in metal or plastic containers, half-filled with water, to rice farms and waterlogged areas. Despite the cessation of any maintenance of the rearing ponds for several years now, the fish continue to breed prolifically in them, such that the current needs of fish for antimalarial activities can still be met. A guidebook on the use of *Gambusia* for mosquito control is available in the Dari language.

Bahrain

Rathor [16] indicates that *Aphanius dispar* is native to Bahrain but has rarely been bred in aquaculture.

Cyprus

In Cyprus, *Gambusia affinis* was introduced in the late 1940s and was present in man-made reservoirs, wells, marshes, ditches, dams, lakes, ponds and even streams. It controlled larval breeding in habitats that were otherwise not amenable to chemical larviciding. It is currently available all over the island country in dams, artificial reservoirs and most stagnant water collections. Since 1980, the fish has been collected in small containers and reintroduced wherever required for antimalaria work although their efficiency in mosquito control programmes is not being monitored (A. Hadjivassilis, personal communication, 2001).

Djibouti

In Djibouti, *Aphanius dispar* has been successfully evaluated to control mosquito larvae around the capital and efforts were being made to extend this strategy throughout the country [17]. During a recent visit in 2000, Beljaev reported that the use of fish for larval control is still practised in Djibouti (A. Beljaev, personal communication, 2000).

Egypt

Egypt was one of first countries of the Region to import *Gambusia affinis*, obtaining it from Italy and Georgia during the 1920s. Following recommendations of the Antimalaria Commission, an integrated approach to control malaria was launched and the introduction of *G. affinis* was an important component of this strategy [18]. Following the DDT era, no further information was available on the use of fish except for their evaluation for the control of culicine larval breeding in wells [12]. Since then the method has been totally neglected.

Islamic Republic of Iran

G. affinis holbrooki were introduced from Italy into the Ghazian marshes, Caspian littoral in Iran during 1922–1930 and after initial technical problems, were successfully used in combating malaria [19]. Beginning in 1966, intensive efforts were made to introduce the fish in the whole country. Around this time Tabibzadeh et al [20] reported that the introduction of *G. affinis* fish in a village marsh combined with house spraying of DDT caused the disappearance of vectors and a great reduction in blood slide positivity rates. The introduction and use of *Gambusia affinis* for malaria control in Iran was reported in 1966 (T.D. Mulhern, unpublished data, 1966). It was also reported that in a hotel garden pool where gold fish (*Carassius auratus*) were present, no larvae were seen compared with considerable numbers of larvae in nearby similar pools without fish. A paper by Ismail Rostami translated from Farsi and summarized by Mulhern mentions malaria control in Iran by biological methods, including, interestingly, the highly successful method of transporting fish in wooden barrels by trucks during the night. Recently Gouya reported that *Gambusia* has been used for the last 40 years in Baluchistan and Sistan provinces and that *Aphanius dispar* are used in Hormozgan, Kahnuj and Kerman provinces (M.M. Gouya, personal communication, 2001).

Iraq

In Iraq, *G. affinis* was introduced in 1954 and is known to have established itself in Dialah, Baghdad and Suleimaniya provinces. In 1979, *G. affinis holbrooki* were found in parts of Basra city in all the irrigation canals and their use in all kinds of water collections in the city was suggested (R. Bahar, unpublished data, 1979). Fish breeding ponds are still in use at least in the northern part of Iraq (A. Beljaev, personal communication, 2002).

Jordan

G. affinis was introduced in Jordan in the late 1950s in the Jordan Valley from where the fish were taken to Syria in 1962 and introduced in a water collection near Aleppo. Fish breeding ponds were established at the Entomological Field Station in Jericho and the fish were later distributed in several parts of the country for malaria control.

Kuwait

The *Anopheles* mosquito species was detected recently in Kuwait. Although the larvivorous fish, *Aphanius dispar*, is native to Kuwait, there has been no record of the use of fish for mosquito control.

Lebanon

G. affinis was introduced in 1944–45 in Nahr Damaver and again in 1962–63 in Nahr Beirut and Nahr Ibrahim, for malaria control. The fish was reported to be present in natural habitats and streams in 1990, but no evaluation has been made in recent times [12].

Libyan Arab Jamahiriya

Little published information is available on larvivorous fish in the Libyan Arab Jamahiriya although *Gambusia affinis* is reported to be present in some lakes.

Morocco

G. affinis was introduced around 1929. The fish were re-introduced in 30 ponds in the late 1950s where it established and eliminated the breeding of anopheline and culicine larvae. This fish has established itself so well that it is being disseminated every year as part of the national mosquito control programme (A. Beljaev, personal communication, 2002). Institutes under the Ministries of Health and Agriculture undertake research work on fish and there are departments to promote the breeding of inland fish (L.E. Abderrahmane, personal communication, 2001).

Oman

Oman has had a rich experience in the use of the indigenous larvivorous fish, *Aphanius dispar* (locally known as *sud*), for malaria control. *A. dispar* is known to reside in a wide variety of habitats (R. Haas, unpublished data, 1979) and is a highly versatile fish that can tolerate a range of environmental and water conditions, including both freshwater and brackish waters. It has been noted that in the age-old underground irrigation channels (locally called as *falajes*), where the fish has been established for a long time, mosquito breeding was unlikely to occur (G.R. Shidrawi, unpublished data, 1976). During special control trials in the Samail area in the interior region of Oman, the introduction of *A. dispar* in water bodies together with mosquito breeding habitat reduction at the beginning May 1976 caused a noticeable drop in the biting rates of the malaria vectors *An. culicifacies* and *An. fluviatilis*, as well as a drop in the malaria parasite rate (G.R. Shidrawi, unpublished data, 1977). Another observation made 2 years later in 1978 confirmed the presence of the fish in deep wells where they were introduced in 1976 (R. Haas, unpublished data, 1979). Currently, *A. dispar* exists in 3500 tanks in the Batinah region and in most *wadis* (valley streams).

Pakistan

The presence of *Poecilia reticulata* and *Oreochromis mossambicus* (earlier reported as *Tilapia mossambica*) is reported in Karachi. *Aphanius dispar* is also reportedly present in the country. Experimental studies on the larvivorous potential of locally available fish have been undertaken [21,22]. Species of *Puntius* are also known to be present in Pakistan and eastwards.

Saudi Arabia

The mosquito larvivorous fish, *Aphanius dispar*, are reported to be found in shallow channels near Riyadh and its larvivorous efficacy was evaluated in two cemented tanks in Riyadh in 1979 [23]. The fish, applied at about 3 fish/m^2 water surface, controlled mosquito larvae successfully. Recently, the presence of this fish was reported in high numbers in Qunfudha and in a valley (Wadi Al Kana) and its use recommended in an integrated approach (G.R. Shidrawi, unpublished data, 1996).

Somalia

A scientifically planned field trial to evaluate the efficiency of *Oreochromis spilurus spilurus* was jointly undertaken from June 1980 to July 1982 in a semi-arid area of the Burao district, Togdheer region, northern Somalia by the Somali government, the WHO Regional Office for the Eastern Mediterranean and the UNDP/WHO/World Bank Special Programme for Research and Training in Tropical Diseases [5]. The fish were brought from permanent streams at Behin and introduced in 16 villages in all man-made, ground-level, cement-lined water tanks (*birkets*), which were the only permanent breeding habitats of the malaria vector, *An. arabiensis.* Another 10 villages, where no fish was introduced, served as the control. The average fish application rate was 1 fish/3 m^2 water surface (size of the fish not mentioned) and the fish had to be re-introduced in some *birkets* that dried up due to scanty rains. The trial conclusively proved that *O. spilurus spilurus* fish were highly effective in significantly reducing vector population and malaria parasite rates. *Oreochromis* species are available in permanent water streams in the mountain range in Somaliland. *O. spilurus spilurus* are occasionally collected from these permanent sources and have been seeded in a nursery in Hargeisa (A.A. Mohamed, personal communication, 2001).

Following the successful demonstration of the impact of *O. spilurus spilurus* as a larvivorous fish in northern Somalia, there was renewed interest in the use of the annual fish, *Nothobranchius,* reported from southern Somalia in rain-filled, semi-permanent water bodies. (The term "annual fish" refers to species that survive during the dry season in the form of drought-tolerant eggs buried in the soil in non-permanent waters until the next rainy season when the eggs hatch into young ones. The eggs of *Nothobranchius* can be collected and transported in damp soil/peat. Ripe eggs hatch within a few hours after the introduction of water). It was reported that *Nothobranchius peters* was found in coastal plains in southern Somalia (R.H. Wildekamp, unpublished data, 1983). In Bur Acaba and Bur Eid highlands covered by thorn bush (Bay region), *N. cyaneus* and *N. microlepis* were found in *wars* (small check dams). In Dur Qalin (Bay region), *N. cyaneus* were found in small pools without vegetation. In Qoriolei-Shalanbod-Genale, flat land in a flood basin of the Shebelli River (Lower Shebelli region), *N. cyaneus* and *N. patrizii* were present in the swamps, roadside ditches, field depressions and irrigation seepages, but not in canals and in the river where *O. spilurus spilurus*, in all probability, eliminated the *Nothobranchius* species. Similarly, in Giohar (Middle Shebelli region) marshes, canals and riverine areas had *N. patrizii. N. cyaneus* and *N. patrizii* were found in swamps and pools in Gelib-Giamma-

Kisimayo-Badada (Lower Juba region). Wildekamp recommended planting both *N. microlepis* and *N. cyaneus* for anti-larval work at 2 fish/m^2 in the southwest of Baidao in the Bay region, although *N. microlepis* was an efficient larvivorous fish species by itself.

Sudan

While the construction of the Sennar Dam on the Blue Nile in the 1920s helped in the development of the Blue Nile basin in Sudan, it unfortunately generated adverse health impacts as well. Faced with outbreaks of malaria and the increased prevalence of schistosomiasis in agricultural workers in the Gezira irrigation scheme in central Sudan, the Blue Nile Health Project was established in 1979, which *inter alia* included the promotion of biological control of disease vectors as part of an integrated approach. A fish fauna survey in 1980 in the canals of the Gezira irrigation scheme showed the presence of *Oreochromis niloticus* and *Gambusia affinis* among the known larvivorous fish species [24]. The study further reported that while *O. niloticus* were abundantly present, the exotic *G. affinis*, which had been specifically introduced in the irrigation system for malaria control, had only a residual presence due to improper management. To eliminate aquatic weeds in the irrigation systems in the basin of the Blue Nile, which supported heavy breeding of mosquitoes and snails, Redding-Coates and Coates [25] had suggested the introduction of the phytophagous species, *Tilapia (Oreochromis) zillii*, available locally in the White Nile system of Sudan. *Tilapia zillii* is known to successfully eradicate weeds as well as co-exist with its sibling, *Oreochromis niloticus*. Information on the food preferences of *G. affinis* and *Oreochromis niloticus* in the Gezira irrigation canals has been reported by El Safi et al [26]. They found that *Gambusia* larger than 25 mm in length were carnivorous and showed a marked preference for mosquito larvae. *Oreochromis niloticus* of less than 150 mm in length were also markedly carnivorous and were reportedly useful for mosquito control.

Syrian Arab Republic

G. affinis were brought from the Jordan Valley and introduced in Syria in 1962 in a water collection near Aleppo. From this original source, the fish were distributed to other areas and natural waters and some hatcheries were established. The fish were found to control the breeding of *An. sacharovi* in drains, streams, lakes and irrigation reservoirs. Sadek, as cited by Zahar [12], recommended that the cooperation of the General Establishment of Fisheries be sought, since a local capacity for breeding and distribution of fish already existed there. *G. affinis* is presently being used in a limited area in the Gahb Valley (Hama Province) to control the malaria vector, *An. sacharovi,* whereas there is no follow up of its presence in other permanent water bodies (S. Karch, unpublished data, 2001).

Tunisia

Zahar [12] provided a review of the use of *Gambusia affinis*, which were introduced in Tunisia in a government sponsored malaria eradication programme throughout the country. Even herbivorous species (names not mentioned) were introduced together with *G. affinis*. The latter is so well established that it is disseminated every year for mosquito control (A. Beljaev, personal communication, 2002).

United Arab Emirates

The United Arab Emirates has moderate malaria endemicity and local transmission of malaria occurs only in the north-eastern part of the country. In certain parts of the United Arab Emirates, mosquito control is undertaken using tilapia (*Oreochromis)* and *Aphanius dispar* in wells, basins and natural water reservoirs where farmers do not want application of insecticides for fear of contamination (W. Takken, unpublished data, 1997). Use of larvivorous fish started on an experimental basis in 1992 and on a regular basis in 1998. *Aphanius dispar* and tilapia fish are found in lagoons, pools, dams, streams, canals (*falajs*), shallow wells and brick-made basins in Abu Dhabi, Al Ain, Ras Al Khaimah, the West Coast, the East Coast and in the central plateau regions (A. Musa'd, personal communication, 2001). In a trial supported by the UNDP/WHO/World Bank Special Programme for Research and Training in Tropical Diseases, conducted from November 1998 to October 1999, these fish were introduced in 2412 basins and 3204 shallow wells and the efficiency of *Aphanius dispar* fish in controlling mosquito breeding was reported to be good.

Yemen

A. dispar is indigenous to Yemen and has been successfully stocked in several *wadis* for vector control. It has been reported in large numbers in various *wadis* such as Banna (Abyan), Toban (Lahj), Mawr (Hodeida) and Warzan in the recent past (unpublished data, M. Suleman, 1999; G.D. Souleimanov, 1999). It was also reported that small freshwater ponds with local fish (species not mentioned) were found free of larvae (N. Durrani, unpublished data, 2000). In the Abyan area of Yemen, *Gambusia affinis* were reportedly introduced earlier, but they were not present on Socotra Island.

5. Mass culture of larvivorous fish

Mosquito control operations require the introduction of a large number of larvivorous fish in aquatic habitats. With the exceptions of guppies, *Gambusia* and *Oreochromis* species, most other fish species do not breed in large numbers in their natural habitats since they require special spawning grounds, egg laying sites and optimum temperature. Because of the low food value of these other fish species, fish farmers do not culture them in captivity as part of inland fisheries. Thus, mass production of these other fish species, often under controlled conditions, is required.

Mass production of guppies and *Gambusia* can be achieved by rearing these fish in specially constructed hatcheries or in naturally available ponds as both fish are viviparous and require no special breeding sites. These fish breed throughout the year and, therefore, large stocks can be produced within a short time.

The mass culture techniques for guppies and *Gambusia* fish are described below and can be adapted for other fish species.

Developing natural ponds/habitats into nurseries

Site selection

Perennial ponds of different sizes are commonly found in rural areas, many of which could be used for mass production of larvivorous fish. In urban and industrial areas, man-made tanks of suitable sizes can be similarly used. The important steps for preparation of natural ponds into hatcheries are outlined below.

Removal of predatory fish

It is important to remove large predatory fish for better survival of the mother stock of larvivorous fish to be raised in the ponds. Predatory fish such as catfish (*Mystus* spp.), snake-headed fish (*Channa* spp.) and freshwater shark (*Wallago attu*) may devour the mother stocks and offspring of larvivorous fish. The various methods for removal of predatory fish are described as follows.

Repeated netting. A fine mesh (3 mm) seine net is generally used for removal of predatory fish by repeated netting. A seine net is the most commonly used fish net and is available in different mesh and sizes. It has floats (hollow plastic balls) fastened on the upper margin and some weights (often iron balls) on the lower side. When fishing in ponds, when the two ends of the net are pulled forward, it presents a horseshoe shape.

Pumping out of water. Total cleaning and drying up of the water body is the most favourable method where small ponds are developed for mass multiplication of larvivorous fish. After pumping out the water, the bottom should be exposed to sunlight for up to a fortnight. The bed of the pond is then treated with powdered quicklime at 250–300 kg/hectare to ensure hygienic conditions and adjustment of soil pH. Lime neutralizes the acidic condition and kills bacteria and fish parasites (optimal pH is 7.0–8.5). After 15 days of lime application, the pond may be refilled with water for fish culture.

Using fish toxicants. Mahua *(Madhuka indica)* oil cakes (MOC) may be used at 2500 kg/ha for removal of the predatory fish. Bleaching powder may also be applied at 500-1000 kg/hectare to kill predatory fish. Two weeks after the application of fish toxicants and the removal of the dead fish, the pond may be used for fish culture.

Removal of aquatic weeds

Aquatic weeds pose major problems during mass culture of larvivorous fish in natural ponds. The most problematic aquatic weeds are the floating weeds, e.g. *Eichhornia, Pistea, Wolffia, Salvania* and the marginal weeds, which are mainly rooted at the margins of the ponds, e.g. *Typha* and *Ipomoea.* Submerged weeds, e.g. *Hydrilla* and *Najas*, are not so problematic. Aquatic weeds can be removed manually from the ponds. Deepening and sharpening the edge of the pond above the water level to reduce the area where marginal weeds appear can check the growth of the marginal weeds.

Removal of aquatic insects

A number of aquatic insects, such as water beetles and water bugs, can harm the fish. They can be removed from the hatcheries by spraying oil–soap emulsion on the surface of the water. After spraying, the water surface should not be disturbed immediately. The emulsion film chokes the respiratory system of aquatic insects and kills them. The emulsion can be prepared and applied by mixing mustard oil or groundnut oil with a cheap washing soap powder in the ratio of 56:18 kg/ha. Emulsion of diesel oil (50 l/ha), water (40 l/ha) and a cheap washing soap (5 kg/ha) has also been reported to kill aquatic insects. Aquatic insects should be removed at least one week before the release of larvivorous fish brooders.

Fertilization

Village ponds generally do not require fertilizers to be added for rearing the larvivorous fish because of their location near the agriculture fields that contain organic manure in the form of human or cattle excreta. Newly excavated hatcheries, however, may be treated with a mixture of nitrate, super-phosphate and muriate of potash before filling with water. The rate of application of fertilizers depends upon the type of soil in a particular area and the proximity to agriculture fields. For details on fertilization of new ponds, see the section entitled, Development and preparation of hatchery pond, later in this chapter.

Design and fabrication of nursery ponds/hatcheries

Site selection

Fertile clay and loamy soils are most suitable for the construction of hatcheries since they have low seepage and good water retention. The following points should be taken into consideration for site selection.

- The land should be fertile; loamy/clay soils are best.
- The pH of the soil should be between 7.0 and 8.5.
- The land should have good water retention capacity.
- Land with a high water table is desirable.
- The site should be accessible, preferably by a vehicle.
- The hatcheries should be close to water sources.
- The site should not be low lying and prone to flooding.
- The site should receive adequate sunlight.

Design and construction

The design of hatcheries should take into account the necessity of catching the fish, and therefore rectangular hatcheries are most appropriate, although any other practical shape may also be designed in consultation with a local civil engineer. While the size of a hatchery may depend upon the number of fish required for larval control, minimum dimensions of 10 m length, 5 m width and 1.5 m depth should be used. This may be constructed through simple land excavation. Usually the excavated soil is used for embankment construction, maintaining a slope of 1.5:1 (horizontal:vertical). However, if the soil is not suitable, a mixture of sand and clay (1:2) should be used to make the embankment compact. Alternatively, a short creeping grass may be planted for turfing of the top and sides of the embankment to prevent soil erosion. The embankment should be repaired if necessary after each monsoon. A permanent water source must be provided. If the source of water is a canal, the water should pass through a small tank fitted with a gate to regulate the inflow of water. The outflow pipe in this tank should have a steel/brass wire-mesh to prevent entry into the hatchery pond of unwanted fish species originating from the canal water.

Where local soils are highly porous, it may be necessary to construct the hatchery with walls or embankments made of bricks and the bottom covered with cement plastering (Figure 2). A drainpipe fitted with steel/brass wire-mesh at the inlet point should be provided to take care of the overflow of water. The site should be adequately fenced. Again, if the water is drawn from a canal, pass the water through a small tank fitted with a regulating gate and screened outflow pipe as explained earlier. If the water is drawn from a piped supply system, strictly avoid chlorinated tap water, but if inevitable, the chlorinated water should pass slowly through a small portable aeration tank provided before the main tank. Fix some stones at the bottom in the corners of a rectangular tank (or along the lower rim of a circular tank) to create a natural environment for the fish.

Note. Where water supply is adequate and water retention capacity of the soil is good, the bottom of the tank should not be cemented. This will allow soil–water contact and therefore better production of fish food.

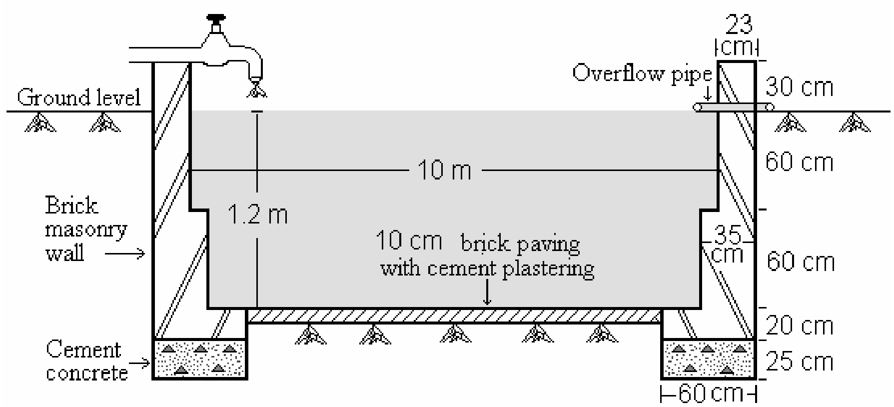

Figure 2. **Design of a hatchery pond**

Stocking and distribution tanks at the periphery

Cement-lined tanks of rectangular (say 5 m length, 2.5 m width and 1.5 m depth) or circular shape can be set up for stocking of fish at the local level for further distribution. The tank may be constructed below ground level through excavation. The tank should be constructed of brick on all sides with a proper foundation and thickness and cement-plastered on the walls and bottom. In arid areas, rainwater-harvesting devices may be provided and the tanks may be only partially covered since sunlight is essential for growth of plankton in water and for disinfection of the hatchery. (See Figure 3.)

Problem of seepage and remedies

Newly excavated hatcheries may exhibit low water retention capacity, especially if the soil is porous/pervious or sandy. The bottom of such sites should be consolidated by repeated rolling and ramming of a layer of 20–25 cm of mud clay or bentonite. It may further be consolidated with a layer of loam for better fish food production. Based on consultations with a local irrigation or civil engineer, plastic sheeting may be required underneath in some situations.

Development and preparation of hatchery pond

Hatchery pond with an earthen bed: In newly excavated hatchery ponds with an earthen bed, apply quicklime (calcium oxide) by hand on the earthen bed at 200–1000 kg/ha on the soils in the pH range of 4 to 7, applying a higher quantity of lime in acidic soils. (Lime neutralizes soil acidity, precipitates colloidal matter such as clay suspended in the water, promotes bacterial breakdown of organic matter and supplies calcium for the growth of plants and fish.) After leaving the hatchery pond as such for one week, fill and maintain a depth of water up to 120 cm, keeping at least 30 cm free board.

Next, apply locally available raw or composted farmyard manures such as cow (or goat/sheep/camel) dung and poultry waste at 1–1.5 kg/m^2. A week later, sprinkle or cast a small quantity (1 kg/50 m^2) of fertilizers, i.e. urea (nitrogen content 45%), single super phosphate (P_2O_5 content 15%) and potash (K_2O content 48–62%) in 9:4:2 proportion during daytime. Allow the pond water to turn greenish over a period of about two to four weeks. Float lotus, lily, hydrilla or any other locally available floating vegetation, on which the fish will spawn and under which the young ones will take refuge from intense sunlight.

Hatchery with walls or embankments made of bricks and the bottom covered with cement plastering: Fill and maintain a depth of water up to 120 cm, keeping at least 30 cm free board. Apply slaked lime (calcium hydroxide) to make the water alkaline (1.5 kg/50 m^2, or until the pH of the water reaches 7–8.5). To prepare slaked lime, place a measured quantity of the quicklime powder in a metal or earthen container and pour in a sufficient quantity of water to make a slurry. The reaction of the quicklime with the water will generate heat. Allow the liquid to cool for a day or two and use. Apply manures or fertilizers in the hatchery as explained earlier for the hatchery pond. Allow the pond water to turn greenish over a period of about 2 to 4 weeks. Float lotus, lily, hydrilla or any other locally available floating vegetation.

Periodic fertilization and pH maintenance of hatchery: Either the above-mentioned manures or an equal ratio of rice bran and coconut/mustard oil cakes (1–10 kg/50 m^2) soaked in water overnight should be applied periodically, depending upon algal growth. (Algae growth is considered optimum when 50 ml water taken from the fish hatchery and filtered through plankton netting yields approximately 2 ml plankton.) Maintain the pH of the water at the level of 7.0–8.5 by further application of slaked lime. In the event that hatchery ponds are excavated in alkaline soils, the pH will need to be lowered. To lower the pH, either the earthen bed of the hatchery or the water should be treated with gypsum (calcium sulfate) powder and the rate of application, depending upon the type of soil, should be determined in consultation with a local soil expert/scientist.

Propagation of the fish

The best time for initiating the mass culture of fish is the spring season (March-April). During this period the young fish attain maturity with the rise in temperature. After attaining maturity they start breeding in about a week's time. (For the incubation period of various species, see Section 3, Table 3). After a hatchery is properly constructed, fertilized and filled with water, about 3000–5000 mature and healthy fish should be introduced into it. There should be an equal proportion of males and females in the mother stock. *Gambusia* produces 200–300 offspring per year. If the survival rate of offspring is about 70% and 5000 mature fish of equal sex ratio were introduced at the beginning, up to 400 000 fish will be produced per year, provided they are periodically netted out, leaving adequate mother stock behind.

Maintenance of fish stocks

No special measures are required for maintaining the fish stocks beyond keeping the growth of aquatic weeds in check and maintaining the required water level in the ponds to avoid drying up the hatchery. Periodic thinning of fish stocks may be required to avoid overcrowding. Guppy and *Gambusia* breed almost year round in the tropics, showing two peak periods, March to May and July to September. Once a good population density is established, the hatcheries are ready to produce a periodic supply of fish for larval control.

Monitoring of fish density

Periodic monitoring of fish density should be carried out to check proper multiplication of the fish. This may be particularly needed when fish are not observed in expected numbers in the hatchery or they are suspected to be infected. Monitoring may be done with the use of a small seine net or a mosquito net. Netting should be carried out in succession at five fixed spots to calculate the average number of fish caught. The number of young fry should also be noted.

Composite fish culture

Experiments have shown that larvivorous fish can be cultured along with food fish, such as the carps (e.g. *Labeo rohita, Catla catla, Cirrhinus mrigala, Cyprinus carpio,* Chinese grass carp and silver carp). Larvivorous fish production can also be linked with edible fish production as an incentive to generate income to the communities. *Oreochromis* act as larvivores and have food value when fully grown.

Protection of the environment

Pollutants and pesticides

It is essential to protect nursery ponds from pesticide pollution (especially pyrethroids, which are toxic to fish), sewage effluent, factory effluents and flooding. High chlorine concentrations in water might kill fish (tolerance to chlorine can be determined for local species by experience, especially if it is intended to introduce fish in chlorinated domestic waters). *G. affinis* is known to tolerate mosquito larvicides applied at their operational rates [27,28].

Environmental impact of exotic species

Some species of fish have been reported to produce adverse ecological impact when introduced into a non-native environment. For example, Myers reported that *Gambusia* destroyed eggs and fry of other fish [29]. Bay, however, did not consider *Gambusia* to cause ecological threat [30]. In north India, 70 out of 122 hatchery ponds were washed away in an unprecedented flood in 1990, but *Gambusia* was not able to colonize new water bodies in the area [31]. Tilapias (*Oreochromis*) are prolific and year-round breeders and are considered a threat to carp cultures in confined waters. Exotic species should therefore be introduced in new areas with caution.

Predators

Fish stocks should also be protected from predatory frogs, birds, water snakes, carnivorous fish, arthropods, helminths, clams, etc. Methods to eliminate predators have been discussed earlier in this chapter.

Disease pathogens and their control

Bacteria, fungi, protozoans, helminths and crustaceans cause the common infections of fish. Some important diseases, their symptoms and possible treatment are summarized in Table 4.

Fish collection and transportation

Larvivorous fish may be captured from the hatcheries by trapping or with the help of seine or mosquito nets of different sizes, as required (see Figures 4 and 5). The method is similar to that of collecting fish fry of edible carps from nurseries and rearing ponds.

Tools

- Small fishing nets (1 × 1.5 × 1.5 m)
- Fine mesh (5 mm) seine nets of various sizes for fishing in large ponds (e.g. 2 × 5 m, 2 × 10 m and 2 × 30 m)
- Plastic sieves, or hand nets of rectangular or circular shape
- Plastic buckets/styropore drums
- Large plastic tubs (100 litres)

Table 4. **Disease pathogens and their control**

Disease	Pathogen	Symptoms	Treatment of infected fish
Bacterial			
Fin and tail rot disease	*Pseudomonas*	Faint white line on the margins on the infected fins which leads to disintegration	Bathe in 1:20 000 copper sulfate solution for 2 minutes
Dropsy	*Aeromonas*	Inflammation of belly, bulging eyes, accumulation of water in the body cavity	Dip in 1–5 ppm $KMnO_4$ solution for 2 minutes or treat nursery pond with 1 ppm $KMnO_4$ solution
Eye disease	*Acromonas*	Eyes become vascularized and later opaque, and the eyeballs get damaged	Bathe in 5–10 mg/litre chloromycetin solution for 1 hour
Ulceration	Bacteria	Bacteria cause ulceration of the body of the fish, exposing the muscles	Disinfect nursery pond water with 0.5 ppm solution of $KMnO_4$, or dip in 1:2000 copper sulfate solution for 2–3 minutes for 3–4 days
Fungal			
Fish mould	*Saprolegnia*	Ulceration of skin and haemorrhaging	Bathe in 3% NaCl, or in 1:3000 copper sulfate solution for 3–4 days, or dip in 1:10 000 malachite green solution for 3 seconds.
Gill rot	*Branchiomysis*	Gill filaments become whitish, dropping off eventually, and fish tend to remain at surface gasping for air	Bathe in 3–5% NaCl solution or 5 ppm KMnO4 solution for 5–10 minutes

Table 4. **Disease pathogens and their control (cont.)**

Disease	Pathogen	Symptoms	Treatment of infected fish
Protozoan			
White spot disease	*Ichthyophthirius*	Whitish cyst on the body, gills and fins	Dip in 2–3% NaCl solution for 2–3 minutes daily for 6–7 days; or apply quicklime at the rate of 300–500 kg/hectare in 2–3 installments over a 2-week period in the pond
Trichodinosis	*Trichodina*	Irritation and respiratory trouble, identified by the fish rubbing its body on the margin of the pond; skin gets bluish-white coating	Dip in 2–3% NaCl solution for 5–10 minutes, or dip in 1:1000 acetic acid solution
Myxosporidiasis	*Myxosporidians*	White or dark red cysts on different parts of the body and internal organs	Heavily infected fish should be killed, others may be treated with 2–3% NaCl solution for a few minutes
Other			
Trematode infections	*Gyrodactylus* and *Dactylogyrus*	Serious infections of skin and gills resulting in fading of colour, dropping of scales, and excessive mucus on fins. When the infection is in the gills, excess secretion of mucus is noticed on the gills' surface	Treat the infected fish in 1:2000 acetic acid solution or dip in 5% NaCl solution for 5–10 minutes. Digenetic trematodes, cestodes, nematodes and acanthocephala are not much cause for concern for the maintenance of larvivorous fish stock in nursery ponds

Collection methods

In large ponds, fish netting may be carried out by two to five persons well acquainted with fish collection and able to swim (see Figures 6 and 7).

The net is held by rope from both the sides of the pond and others keep the net intact in the middle and move across the hatchery, thus pulling the net with entrapped fish to a shallow corner. On reaching the margin, the net is kept in water and the fish are transferred to plastic buckets with the help of a soft plastic sieve to avoid injuries. The fish are then further transferred to big plastic tubs/drums for transportation.

In constructed hatcheries and stocking tanks, fish can be collected either by the above method or by using hand nets with a long handle.

Transportation

The larvivorous fish can be transported by one of the following methods.

In open containers: In this method fish are transported in open containers of different sizes depending upon the requirement of the fish. This method is suitable for short distances, say about 50 km (1–2 hour drive) from a mother hatchery. Fish can be transported by this method safely as the open containers allow exchange of atmospheric oxygen in water.

Generally, the following types of containers are used in this method:

- large plastic drums (50–100 litre capacity), or
- galvanized iron box lined with plastic sheet mounted on a jeep or a truck.

The fish are transferred into these containers with the help of small buckets in early morning hours. These should be filled with cool water (20–25°C temperature) to about two-thirds level. The containers should be covered properly with fine nylon netting and placed in a fixed position in the vehicle (see Figure 8). The following precautions should be exercised during transportation of the fish.

Overcrowding should be avoided to prevent mortality during transportation. No more than 2500 fish should be accumulated in one plastic drum of about 100 litre capacity. No more than 50 000 fish should be transported in an open container kept in a jeep trailer. The vehicle should be driven steadily to avoid overflowing of the water from the containers. Avoid sudden stops to prevent injuries to the fish. Short distance transportation should be in the early morning hours to avoid heating of the water under direct sunlight. Young hatchlings should not be transported because they will not survive the stress of transportation. Precautions should be taken during the introduction of fish in water bodies. To prevent sudden shock to the fish, take water from the new habitat and mix it with the water in the container carrying the fish. Allow the fish to adapt and release them gently into the new water body.

In closed containers: When fish need to be transported to distant places, closed container systems should be used. Materials required include polyethylene bags, chlorine-free water, tin containers of 15 litre capacity, oxygen cylinder, thick thread and paper, etc.

Fish packing procedure and precautions

Step 1: The fish are collected from the hatchery and kept for 3–4 hours in chlorine-free water in a net (1 × 1 × 1 m) for conditioning. Conditioning of fish allows them to empty their stomachs and is essential because the fish excrete a lot of ammonia and other nitrogenous wastes. Decomposition of these products may cause high mortality in a closed container system.

Step 2: Leak-proof polyethylene bags are filled with chlorine-free water to two-thirds level. One bag is kept in each tin container (or canister) or cardboard box lined with paper (or newspaper) (see Figure 9). For long distance transportation cool water (20–25°C) should be used (sometimes ice is used to achieve this temperature).

Step 3: 300–500 fish are introduced carefully into each bag, using plastic sieves or a hand net, avoiding damage to the fins of the fish.

Step 4: Oxygen is added to make the polyethylene bag fully blown like a balloon and the bag is closed tightly for transportation.

Step 5: The bags are tightly closed with thick cotton thread and the lids of the containers are closed properly. The tins are now ready for transportation either by motor vehicle, train, or air, as the situation demands. Direct sunlight on the containers should be avoided to prevent heating.

Step 6: At the release spot, the polyethylene bags are removed from the container and put in the receiving water for about 15 minutes to adjust the temperature. Thereafter, the polyethylene bags are filled with water taken from the local water body. After waiting for a few minutes, the bags are immersed in the new water and the fish are allowed to escape.

6. Monitoring and evaluation of the impact of fish

Types of larval habitat

The larvivorous fish can be used in a variety of aquatic habitats for control of mosquito breeding:

Man-made habitats: Water tanks (domestic and industrial), lakes, fountain pools, cattle troughs, swimming pools, cisterns, ponds, shallow wells, waste ditches, husk pits, drains, water storage tanks at construction sites, seepage water pools and water reservoirs of different types, especially in desert locations, irrigation cisterns and canals, shallow ponds, small dams and rice fields.

Natural habitats: Ponds, lakes, riverbed pools, slow moving small streams, swamps and temporary water collections during rainy season.

Fish release and monitoring

Selection of an appropriate larvivorous species should take into consideration the ecology of vector breeding sites (temperature, pH, salinity, pollutants, chlorine concentration). A thorough geographical reconnaissance should be carried out to determine the type and location of breeding habitats. The rate of release of fish depends upon the type and size of fish and the ecology of larval habitat. Usually *Gambusia* fish are released in larval habitats at about 5 fish per square metre of water surface and replenished thereafter as required. *Oreochromis spilurus spilurus* released at the rate of one fish per 3 m^2 surface in cemented reservoirs (*birkets*) in Somalia provided a good degree of larval control.

It is necessary to periodically check the presence of fish in mosquito breeding sites. Fish may be reintroduced periodically, as necessary, from the fish rearing ponds or permanent breeding grounds. In reservoirs where there is a risk of drying up, such as in Somalia, provisions may be made to allow the fish to survive during the dry periods (e.g. by excavating a deep hole in a corner).

Evaluation of the use of fish as a method for mosquito control

The decision to use larvivorous fish in a vector control programme should involve the examination of the following issues to ensure its operational efficiency and sustainability:

- use of fish alone as a method for mosquito contol;
- combined use of larvivoous and phytophagous fish for mosquito contol;
- use of fish as a component of an integrated contol pogramme;
- implementation of fish pogramme by community participation;
- cost-efectiveness of the larvivoous fish pogramme; and
- availability of local capacity and capability to oganize a fish-based pogramme and determination of training needs of the anti-malaria staf on the use of fish.

If the operational potential of a fish species needs to be tested, a study should be designed to assess the following:

- impact on the mosquito densities;
- impact on malaria incidence/morbidity;
- social acceptance of the use of fish and needs for health education; and
- cost-benefit analysis of the operational use of fish.

For further details on the requirements of study designs, implementation and monitoring of impacts, see Annex 2.

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7. Community participation and intersectoral approach in integrated vector control

Introduction

Community and intersectoral participation are considered important elements of an integrated disease control strategy. It is essential to link the malaria control programme with community participation [32,33]. Several recent studies have reported that the sustainability of successful anti-malaria programmes involved cooperative networking between the government health sector and the community [34,35].

It is necessary to inform local communities about the problem of vector-borne diseases and the benefits of the use of larvivorous fish, both as a larval predator and as a human food source. Information and education may be required in communities, especially among schoolchildren and women, who are often not aware of the value of fish as a source of food or as an agent for elimination of aquatic weeds in village ponds. In an experiment in Somalia [5], it was possible to encourage the communities to grow tilapia fish in cemented *birkets* for vector control and to eat them when fully grown. In China, grass carp and common carp are used to control mosquito breeding and additionally provide nutritional and commercial benefits [36]. In Kheda (Gujarat, India), several village councils cultured guppy fish along with Indian carps and the money generated was used for village development. In southern India, removal of floating vegetation from local water bodies benefited the community in reduction of mosquitoes and in the commercial manufacture of paper out of the vegetation removed [37]. Following good health education campaigns in Panaji and Ahmedabad cities in India, people collected guppies in small polyethylene bags and introduced them into household water storage tanks.

Possible activities in which communities can participate

For the successful implementation, monitoring and sustainability of a mosquito control programme involving the use of larvivorous and food/phytophagous fish, local communities, community leaders, schoolchildren, women's groups, peoples' representatives and fish cooperatives can participate in one or more of the following suggested activities.

- Provide support in the identification of suitable sites for setting up fish hatcheries at the local level.
- Provide human resources, material and logistic support in setting up the hatcheries.
- Allow the use of existing ponds or tanks that are suitable for development as hatcheries.
- Ensure the supply of water into the hatcheries, if necessary
- Provide locally available composted manures for periodic fertilization of the hatcheries.
- Maintain the physical condition of the hatcheries.
- Help encourage sociocultural acceptance of the use of fish as a vector control tool and as a source of nutrition.
- Maintain fish in domestic and local waters following introduction by anti-malaria staff.
- Collect fish from the local hatchery for their own use and participate in the local distribution of fish and in the provision of fish to others who might need them for replenishment.
- Identify larval breeding grounds for fish introduction.
- Participate in removal of weeds/vegetation from the weed-infested ponds and other local waters, thereby rendering these weed-cleared waters suitable for fish introduction.

Intersectoral cooperation

Promotion of the use of fish in an integrated disease control programme requires efforts at the national, international, sub-district and village levels. In view of the cross-border dimension of mosquito-borne diseases, countries in a region should cooperate in dissemination of technical information, exchange of fish species (after taking into consideration ecological and environmental effects of the introduction of exotic species), seconding staff in intercountry training and developing consensus for greater use of biological control agents in an integrated disease control programme.

At the country level, there is a need to create greater awareness about the use of simple, economical and environmentally safer methods of mosquito control through mass media campaigns, advocacy workshops and school education. The anti-malaria programme should examine the scope of the use of fish for mosquito control, identify governmental and non-governmental organizations that can cooperate in the promotion of the use of fish, develop/disseminate relevant literature in national/local languages, organize training and advocacy workshops at different levels and set up facilities at national, regional and local levels for manpower development.

Logistically, it is always relevant to set up fish hatcheries at the peripheral or village level. This requires cooperation of various agencies operating at the local level, including:

- ❖ close liaison with local fisheries personnel and cooperation for:
  - – taxonomic identification of local fish species
  - – competent management of hatcheries
  - – estimation of fish densities
  - – management of fish disease
  - – local techniques of transportation
  - – promotion of composite fish culture
  - – use of existing fishery ponds for larvivorous fish culture;
- ❖ cooperation of personnel from the agriculture sector for de-weeding techniques, local supply of manure, etc.;
- ❖ cooperation of the irrigation sector for release of fish in canals, supply of water into hatcheries, etc.;
- ❖ cooperation from public works or civil engineering departments for local design and construction of hatcheries, fixing devices to harvest rainwater for the hatcheries in arid zones, etc.;
- ❖ cooperation of educational institutions for advocacy for their suggestions for local advocacy needs and for the support of schoolchildren in actual implementation of the fish programme;
- ❖ support of mass media agencies for information, education and communication;
- ❖ involvement of nongovernmental organizations, development aid agencies, women's groups;
- ❖ cooperation of and networking with, village councils.

Design and implementation of a community participation programme

Socioeconomically weaker communities may not perceive mosquito-borne diseases as an issue of immediate concern. Therefore, concerted efforts are required to ensure active participation of stakeholder communities in the integrated management of mosquito-borne diseases, especially using fish. There is no single approach to achieving community participation in disease control [36]. The process of design and implementation of a fish programme in a given situation would most often be of a generic nature. Although considerable details on the possible ways to solicit community and intersectoral participation have been discussed above, general guidelines to solicit community participation in a fish-based programme are suggested below for consideration of the anti-malaria personnel and the communities.

Baseline data collection or preparatory phase

It is suggested that during the preparatory phase of the project/programme, community and intersectoral participation should be solicited along the following lines:

1. Select the project area(s) where it is proposed to introduce the use of fish as an element of an integrated control strategy. The selection of the area(s) will depend inter alia upon considerations of ecology, vector biology and operational logistics.
2. Identify agencies, institutions and community groups at national, regional and village/local levels that can support the project.
3. Assess community knowledge, attitudes, beliefs and practices.

   In the project area(s) where it is proposed to implement a larvivorous fish-based intervention, assessment should be made inter alia of:

- ❖ community knowledge, attitudes, beliefs and practices related to mosquito-borne diseases and their control;
- ❖ morbidity caused by mosquito-borne diseases and the resultant economic burden;
- ❖ community attributes (education, economy, etc.);
- ❖ experiences with pisciculture;
- ❖ fish-eating habits of the people;
- ❖ knowledge of pisciculture methods/techniques;
- ❖ knowledge on the availability of fishing gear (nets, etc.), medicines for the treatment of fish diseases, fertilizers, etc.;
- ❖ willingness to participate in a fish programme;
- ❖ capacity to contribute in any way (financial, workforce, materials such as manures, construction materials, etc.);
- ❖ cultural factors in favour of, or against, the use of fish;
- ❖ water storage practices adopted by the communities; and
- ❖ assessment of the suitability of local/domestic waters for fish introduction.

The following techniques can be employed in organizing a survey on community knowledge, attitudes, beliefs and practices:

- questionnaire-based quantitative survey with the help of socio-anthropologists;
- qualitative assessment based on focus group discussions with community leaders, prime movers, support groups, aid agencies and nongovernmental organizations.

4. Identify community advocacy needs based on the survey and develop and implement an appropriate information, education and communication programme. A participatory learning approach would be a useful additional technique in this programme and the communities would appreciate being shown mosquito larvae, fish and a demonstration of the fish preying upon the mosquito larvae. This would also be a good opportunity to explain health, nutritional and commercial benefits of the fish programme.
5. Set up fish hatcheries in the project area to mass rear fish if no hatchery exists already. Decentralized rather than central hatcheries would facilitate local and easy availability of the fish. If locally existing ponds are not suitable for mass breeding of fish, hatcheries should be constructed as previously explained.
6. Undertake a geographical reconnaissance survey with the help of the local community and identify mosquito larval habitats suitable for fish introduction. Also identify if local waters are infested with weeds and whether it is necessary/feasible to introduce phytophagous fish for the elimination of floating weeds.

Intervention phase

1. Introduce fish into larval habitats in the intervention area(s) by involving the local community. The selection of intervention communities needs to be determined by entomological and epidemiological considerations.
2. Continue with the advocacy and information, education and communication programme, if necessary. (See Figure 10.) Discuss the positive impacts of the fish programme. Assist communities if a problem arises in the continuation of the use of fish, or if the netting of extra fish is required. For example, in some villages near Hargeisa in Somaliland, a

local nongovernmental organization introduced *Oreochromis* fish in *birkets*. After a few months the fish multiplied in large numbers but the local people did not know what to do with the fish (they had never previously caught or eaten fish). The nongovernmental organization was of little help since they lacked the technical know-how, thus a very useful programme ended in the very next year of implementation.

3. Develop a mechanism for periodic inspection of the hatcheries, domestic and local waters for the presence of fish. Replenish the fish, if necessary.

8. Training

Training of the staff involved directly in mosquito control operations is a prerequisite for successful implementation of biological control measures as part of the integrated disease vector control programme. There are three main components of such training.

1. *Identification of key trainees*

Personnel with a basic degree in biological science should be identified at each level in the malaria control organization by the national, regional and local managers and trained on various aspects of the use of larvivorous fish for vector control.

2. *Identification of trainers and training institutions*

In countries where training institutes impart training in entomology, vector control and epidemiology, a component on larvivorous fish can be added to the course curriculum. In addition, the services of national or international experts or consultants could be obtained for training of the trainers. The national, regional and local managers in malaria control organizations should identify potential trainers.

3. *Development of training course content*

An organized training programme requires the development of a suitable training content, preferably in the local language. Training content should include the following components as a minimum:

- ecology of vector species and their habitats;
- ecology, biology and distribution of known or potential larvivorous fish species;
- complete information on construction of hatcheries and preparation of natural ponds/tanks for use as hatcheries;
- propagation of larvivorous fish and maintenance of fish stocks;
- transportation of fish and their application in larval habitats;
- monitoring of impact;
- environmental impact of fish;
- precautions during handling of fish;

- fish density determination;
- management of environmental impact of the fish;
- composite culture of larvivorous fish with food fish;
- entomological and parasitological monitoring and data recording;
- community participation.

9. Areas for operational research

Operational research is suggested in the following areas:

- comprehensive studies related to the biology of various larvivorous fish;
- development and use of advanced technology for mass production of larvivorous fish in captivity;
- development and use of hardy strains of known larvivorous fish through genetic manipulation to enable them to withstand extreme environmental factors, such as in temperature, pH, pollution and salinity;
- production of mono-sex sterile populations of larvivorous fish for use in exceptional situations to prevent multiplication and overcrowding of fish;
- development of mixed culture techniques;
- development of advanced methods for safe transportation of fish;
- study of the ecological impact of larvivorous fish on other fauna and flora;
- role of community participation in promotion of larvivorous fish; and
- cost-efectiveness analysis of the operational use of larvivorous fish in countries.
- study of the population dynamics of the vectors and the impact of the use of fish on vectorial capacity and disease transmission.

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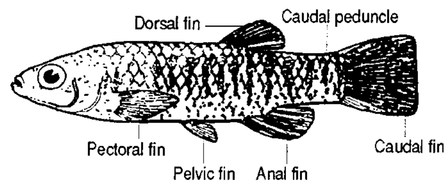
Annex 1

Fish biology, ecology, distribution and characteristics

Known larvivorous fish in the Eastern Mediterranean Region

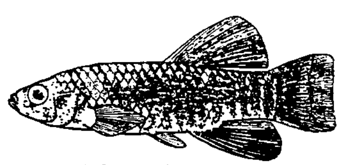
The morphological and other salient features, distribution and mosquito control potential of the known larvivorous fish in countries of the Region are described below (also see Table 3 and Figure 1, Chapter 3). A diagram showing the morphological features of a typical fish is given below to assist the reader in understanding the features of the species.



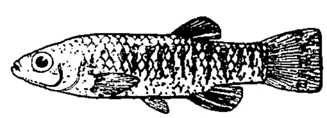
Labeled diagram of a fish

Aphanius dispar

Aphanius dispar is widely distributed along the east coast of the Mediterranean, coastal regions of Bahrain, Djibouti, Egypt, Islamic Republic of Iran, Iraq, northern Somalia, Sudan and the Arabian Peninsula (Oman, Saudi Arabia, Yemen), up to Pakistan and the west coast of India where, historically, it is well known for its larvivorous efficacy [17,23,38,39,40]. It is closely related to genus *Aplocheilus* but differs chiefly in having the dorsal fin in advance of the anal fin. It measures up to 7.5 cm in length. Males are greenish yellow with a black spot just above the pectoral fin and has reticulated markings of brown on black on the sides. The anal fin has an orange-red anterior part while the posterior part has 4–5 black vertical bars. The tail fin is yellowish with 2–3 dark crescent bands. Females are smaller than males. They are uniformly silver in colour with 7–12 distinct black



Aphanius dispar (male)



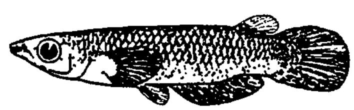
Aphanius dispar (female)

bands on each side.

Aphanius can tolerate high salinity, therefore it is suitable for brackish as well as fresh waters, stormwater drains, cesspools, etc. It can tolerate a wide range of environmental and water conditions (salinity, pollution, darkness, etc.) and thrives well in tropical waters (16–26°C). It is also found in oasis pools with hypersaline to fresh water. It is a year-round prolific breeder, voracious larvicidal and is highly agile. In countries such as Oman, where it is found locally, it should be used for vector control, since indigenous species are well adapted to local biotic and abiotic conditions. This species has been reported present in small numbers in a Mogadishu fish nursery, where the original specimens were reportedly collected from northern Somalia near Scusciuban. Because of its ability to tolerate a wide range of salinity, it is suitable for vector control in coastal areas. Through a randomized, controlled trial, Fletcher et al [6] successfully demonstrated the efficiency of this species in the control of mosquito larvae in a port city in Ethiopia.

Aplocheilus *spp.*

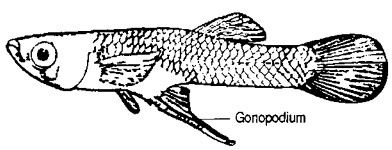
Commonly known as killi-fish, these are small and active surface feeders that mainly inhabit fresh waters and also brackish waters of moderate salinity. Species, viz. *A. blockii*, *A. lineatus* and *A. panchax,* are found in India and Pakistan. It is an efficient larvivorous fish among indigenous larvivorous species. It is an egg-laying fish and the *A. panchax* produces adhesive eggs that attach to aquatic vegetation. The eggs hatch in about 15 days and the fish matures in about 4 months. These fish are small (4–10 cm in length), with an elongated body; males are coloured and the mouth is superior with a long pointed snout. A third eye-like shining silvery spot in the middle of the head is a characteristic feature of this fish, thus making it easily distinguishable from other local fish. The lack of a sex organ (gonopodium) distinguishes it from the guppy and the *Gambusia*. Scanty populations are found along edges of ponds, lakes, slow moving streams, canals, etc. These fish are suitable for introduction in ornamental tanks, fountains, ponds, swamps, wells, reservoirs and overhead tanks.



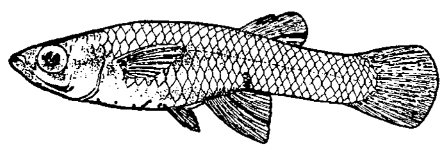
Aplocheilus panchax

Gambusia affinis

The mosquito fish, *Gambusia*, is a top-feeding minnow and is a known good larvivorous fish with wide distribution in countries in the Eastern Mediterranean Region. It is unique in its global distribution and is small, tiny, grey or greyish black, measuring up to 4–6 cm in length. *Gambusia* has been used in many parts of the world to control mosquito larvae. The fish occupies some curious habitats, such as tunnels, abandoned pools, ponds, rainwater pools, stagnant rice fields, etc. It is viviparous (lays young ones and not eggs), breeds prolifically and requires no special egg-laying site. A single female can produce 200–300 offspring in a year. It can be mass-produced easily. It seems to operate most efficiently at a density of about 1250 fish/ha and can efficiently check mosquito breeding in rice fields when introduced at the rate of 250–750 fish/ha [41]. This fish is suitable for low temperature areas, as it is known to survive subzero temperatures. The female attains sexual maturity in 90 to 100 days. Fertilization by males is internal and the female carries eggs in an incubating pouch until they hatch as tiny fish. The gestation period varies from 28 to 30 days depending on temperature. A black-coloured gravid spot in front of the anal fin identifies the brood-female. When the colour of the spot deepens, the birth of the young ones becomes imminent. Gradual maturation of females can be noticed easily. The size of the female is a significant feature when selecting breeding places suitable for its growth. This fish has proved useful in primary or sole-source mosquito breeding sites. It is reputed to have shown potential to reduce mosquito populations throughout the world.



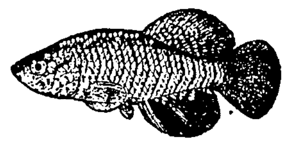
Gambusia affinis (male)



Gambusia affinis (female)

Nothobranchius *spp.*

The annual fish, *N. patrizii, N. peters, N. cyaneus, N. microlepis* and *N. guentheri,* are found in southern Somalia and *N. peters* is also found in the higher plains of southern Sudan. In 1941, Vanderplank reported the value of *Nothobranchius* and *Barbus* species as indigenous anti-malarial fish in east Africa [42]. *Nothobranchius peters* has also been reported in coastal plains in southern Somalia and in higher inland plains in southern Sudan. The fish (normal size 2.6–5.4 cm) breeds on low permeability black cotton soil with high humus, such as in swamps. Annual fish, as the name suggests, are adapted to life in temporary waters by producing drought-resistant eggs that lie buried in the soil during dry periods and hatch into young fry on the arrival of water after rains. The eggs may be collected and transported in a damp condition. When introduced into water, ripe eggs hatch within a few hours. Young ones are hardy and mature rapidly. Realizing the importance of this species, a comprehensive report on the *Nothobranchius* spp. was developed [43].



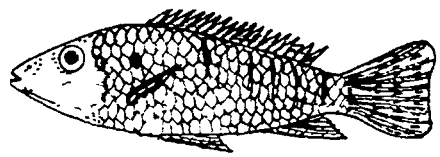
Nothobranchius patrizi

Oreochromis *spp.*

The most promising species of this genus for vector control are *O. spilurus spilurus* and *O. zillii* (in Somalia), *O. niloticus* (Sudan) and *O. mossambicus* (Pakistan). *Oreochromis* (family Cichlidae), commonly known as tilapia fish, are indigenous to east Africa and Somaliland and are represented by more than 20 species. It has a terminal mouth and is an omnivorous or even carnivorous fish (cannibalism occurs during food shortages). This genus was formerly known as *Tilapia*

Oreochromis have 14–17 dorsal spines, 10–13 dorsal soft rays, 3 anal spines, 9–10 anal soft rays, and scales on the cheeks in 2–3 series. Females and immature males present a yellow-buff background colour with a series of mid-lateral blotches and a more dorsal parallel series of blotches. Mature males become golden-yellow with distinct bright blue areas on the dorsal, anal and pelvic fins and have orange or red dorsal lappets. *Oreochromis* either spawns on substratum or the males carry the eggs in their mouth for parental care. Optimum temperature for breeding is 18–28°C. Growth of the fries is rapid as long as there is food. Eggs are not resistant to desiccation. It can tolerate organic pollution, can survive in the temperature range of 10–40°C and has a variable tolerance to salinity and water hardness. *O. nilotica* is reported to tolerate high chlorine and low oxygen levels [44] but the chlorine tolerances of other species need to be established. *O. mossambicus* is also reported to tolerate high organic pollution [45]

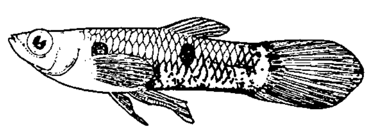
It can attain up to 20 cm in length and can be a food fish. Because of its high reproduction rate in tropical waters and its carnivorous habits, this fish can eliminate other fish in the aquatic ecosystem. Therefore, these fish should be used in confined waters and should not be introduced into new areas where other potential indigenous larvivorous species are present, such as in southern Somalia where the annual fish, *Nothobranchius*, is indigenous. In Somaliland, *Oreochromis* fish were reported to be present in the permanent streams in Behin (100 km north of Burao) and in Lafaroug (200 km on the way to Hargeisa from Burao). Very recently these were collected from streams near Burao and were seeded in a fish nursery tank in Hargeisa town.



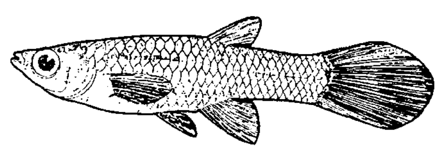
Oreochromis spilurus spilurus

Poecilia reticulata

Poecilia reticulata is a native species of South America. Commonly known as the guppy fish, it was introduced to British India (now Bangladesh, India and Pakistan) from South America in 1908 for mosquito control and is now found in ponds, canals, tanks and ditches in rural and urban areas throughout Bangladesh, India and Pakistan. It is an efficient mosquito control agent with characteristics similar to *Gambusia.* The length of the female ranges up to 4 cm and the males are smaller (2 cm) and brilliantly coloured. At optimum tropical conditions (water temperature 24–28°C), the fry attains maturity in 5–6 weeks and it starts producing offspring at 10–12 weeks of age [46]. The interval between two successive broods ranges from 4–12 weeks (mean: 6 weeks). It is a very prolific breeder and a gravid females gives birth to about 20–50 hatchlings up to four times in a year. Thus, on average, it can produce up to 200 offspring per year. Guppies cannot tolerate temperatures below 10°C. They can withstand moderate organic pollution, and thus are highly suitable for mosquito control in urban drains, polluted waters and wastewater pools, in addition to freshwater habitats.



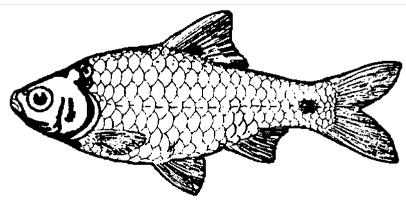
Poecilia reticulata (male)



Poecilia reticulata (female)

Puntius *spp.*

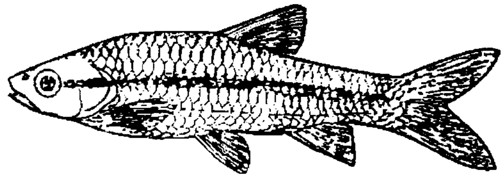
Puntius sophore fish are found in Afghanistan, Pakistan (Sind, Peshawar, Jhelum drainages), and eastwards. *P. ticto* is reported in Sind. These fish are moderately elongated and have a compressed body and rounded abdomen. *Puntius* (earlier known as *Barbus*) fish exhibit a wide range in size (2.5–30 cm length), comprising an assemblage of diversified forms. The larger forms have food value, whereas the smaller forms such as *Puntius ticto* (10 cm) and *P. sophore* (12.5 cm) are hardy and have comparatively little food value. They feed on crustaceans, insects and plankton. Spawning takes place during the monsoon season. About 150 eggs are laid in batches of about 20 at a time. The eggs hatch in about a day and fry are free-swimming the next day. They are found in shoals in confined, shallow and marginal waters of rivers, freshwater ponds, pools, canals and perennial water bodies and are also useful for introduction in rain pools and slow streams. Low population densities may be maintained in carp-culture ponds for mosquito control.



Puntius sophore

Rasbora daniconius

This species is native to India and Pakistan and is reported to be present in Oman and possibly in other Eastern Mediterranean Region countries east of Oman up to the Indian border with Pakistan. *Rasbora* can be distinguished from other closely related genera by its symphysial knob and the absence of barbicles. *Rasbora daniconius* is the most common species of its genera. It attains up to 15 cm in length and can be identified by a dark blue lateral band extending from its nostril through the opercle to the base of its medium caudal rays. Its sides are silvery, its belly is white and its dorsal, anal and caudal fins are orange in colour.



Rasbora daniconius

These fish are widely distributed in tropical areas (water temperature: 24–26°C) in freshwater ponds, hill streams, rivers, ponds, canals, inundated fields and tanks with abundant aquatic vegetation. It is suitable for introduction in borrow pits, ponds, tanks and fountain pools. It is a surface feeder and feeds mainly on aquatic insects and detritus.

Known phytophagous and malacophagous fish in the world

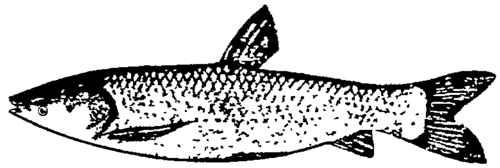
Phytophagous fish

Very often, water bodies are infested with aquatic weeds, which provide shelter to mosquito larvae and snails and provide a physical barrier against natural predators, such as larvivorous fish. Certain species of fish that eat large amounts of aquatic weeds (known as phytophagous fish) can be used to eliminate the algae and other weeds and, thus, complement larvivorous fish in controlling mosquito larvae in aquatic ecosystems. Some of the phytophagous fish species that have been most investigated are:

- Chinese grass carp, *Ctenopharyngodon idella* (Valenciennes)
- Tilapia fish, *Oreochromis zillii* (Chichlidae)
- Giant gourami, *Osphronemus goramy* (Lacepede)
- Chinese silver carp, *Hypophthalmichthys molitrix* (Valenciennes)

Ctenopharyngodon idella

This is a natural inhabitant of China and a small area of Russia and is commonly known as white amur. It has been introduced in a large number of countries for culture as a food fish. It is an efficient phytophagous fish and is reported to grow up to 120 cm in length and 32 kg in weight [47]. When applied in over 750 ponds at the rate of 50 fingerlings/100 m^2 in monoculture (or 20/100 m^2 in polyculture with common carps), they controlled weeds successfully [48]. In Turkmenistan, this species was introduced in the Kara Kum Canal to assist in the control of *Anopheles pulcherrimus* and *Culex* species.



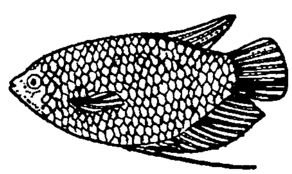
Ctenopharyngodon idella

Oreochromis zillii

This has been used in East Africa to eradicate aquatic weeds and can co-exist with *O. nilotica* [24]. The irrigation schemes in the basin of the Blue Nile are infested with weeds and support heavy breeding of mosquitoes and snails. In 1981, the introduction of the phytophagous species, *Tilapia (Oreochromis) zillii*, available locally in the White Nile system of Sudan, was suggested instead of the exotic species, *Ctenopharyngodon idella*, the Chinese grass carp [25]. *Oreochromis zillii* is known to eradicate weeds successfully as well as co-exist with its sibling, *Oreochromis niloticus*. *Oreochromis zillii* is native to Egypt, Jordan, Lebanon, Morocco, Sudan and Tunisia. It has been introduced in the Islamic Republic of Iran, Saudi Arabia and the Syrian Arab Republic. It grows up to 40 cm in length and weighs up to 300 g. It prefers shallow, vegetated areas and the fry are common in marginal vegetation.

Osphronemus goramy

The giant gourami (*O. goramy*) naturally occurs in fresh waters of Cambodia, Indonesia, Malaysia, Thailand and Viet Nam and is cultured in many other countries. Adult gourami subsist mainly on aquatic vegetation and can turn omnivorous.



Osphronemus goramy

Hypophthalmichthys molitrix *(Valenciennes)*

This species, also commonly known as the Chinese silver carp, is a plankton feeder, but also thrives on decayed macrovegetation and detritus.

In Morocco, where eutrophication was a major problem in drinking water reservoirs, the Chinese silver carp eliminated algae in a reservoir near Rabat in 1987, in a UNDP-supported experiment.

Malacophagous fish

In standing aquatic ecosystems where snail populations occur, certain fish species have been shown to eliminate snails together with their supporting vegetation (for example, Chinese grass carp in Sudan). Potential malacophagous fish are: *Ctenopharyngodon idella*, *Notopterus, Tetraodon, Heteropneustes, Etroplus, Synodontis* and *Carassius*.

Annex 2

Requirements of study design and implementation

The following steps should be considered in the development of a study protocol and in the conduct of a field trial for the evaluation of the operational efficiency of the use of fish for mosquito or vector control

1. Objectives

According to local needs, the objectives of the study should be decided as discussed earlier in Section 6.

2. Selection of the study area

Careful consideration should to be given to the choice of a study area so that ecological conditions are most favourable for the use of fish (e.g. the mosquito breeding areas are limited or confined, such as in arid or urban/industrial areas, or the areas are isolated like islands). The study area must be accessible to field staff collecting reports of vital events and other data on the study communities, to their supervisors, to the teams conducting cross-sectional surveys and to the staff conducting verbal autopsies. The local community should be cooperative and the social situation reasonably stable. If fish migration rates are too high, losses to follow-up will be substantial and it may be difficult to obtain reliable estimates of morbidity or mortality.

It is an advantage if experienced field workers are already available in the area, otherwise they will need to be recruited and trained. Access to necessary clinical, laboratory and data-handling facilities will be needed. Appropriate entomological, social science and other disciplinary expertise should be available, as required.

3. Background information on study area

It is desirable to collect background information of the study area as follows.

Epidemiological information

Information on the epidemiology of malaria in the proposed study area is an important element in the assessment of the suitability of a particular location for the conduct of a larvivorous fish trial. In the protocol, data should be presented to demonstrate the contribution of malaria to morbidity and/or mortality in the study area. As far as possible, these data should be derived from existing sources. These may include: the number of febrile patients with malaria and, in addition, as a proportion of all fever cases whose blood slides were collected through active surveillance; the number of outpatient attendances in health facilities and the proportion with a diagnosis of malaria; the total number of inpatient admissions and the proportion with malaria; and the number of all deaths and those from malaria. An indication should be given of the proportion of microscopic diagnoses of malaria rather than clinical malaria cases alone. Also, an estimate of the catchment population served by the hospital or clinic would be useful to enable rates to be estimated.

Apart from the routinely collected data, which may be of poor quality and difficult to interpret, information based on special surveys that might have been carried out in the area may be given. Such data might include parasitaemia rates and densities at different times of the year and clinical data, for example, on the prevalence of the splenomegaly in children, or on the frequency of clinical episodes of malaria. To decide which age groups of the population will be suitable for the monitoring of the impact of the introduction of fish, the epidemiological information should be presented, if available, to indicate age-specific malaria rates. Accordingly, any available data should be presented separately for the 0–1, 2–4, 5–9, 10–14 and 15–19 age groups and adult age groups.

Anti-malaria measures in the area

Information should be provided on the past and current anti-malaria measures in the area. This can include, for example, insecticide spraying programmes, the use of mosquito nets, the use and availability of larvivorous fish, domestic usage of insecticides or repellents, the availability and reach of health care services for diagnosis and treatment of malaria including severe and complicated cases, the presence of private health providers and the availability of anti-malarial drugs. Use of insecticides in agriculture should also be indicated.

Entomological information

Provide any existing information on malaria vectors, types of breeding habitats, vector behaviour (resting, biting, etc.), host preferences, seasonality and their susceptibility to insecticides.

Demographic, cultural and other information

Provide information on the size of the population or the communities, whether these are dispersed as hamlets or are nucleated and the stability of these communities. Provide some indication of the socioeconomic status of the population. Indicate the literacy level, language of communication, presence of nongovernmental organizations, fisheries extension services, cooperative societies and any cultural issues that may influence the large-scale use of fish in the area (e.g. aversion to the use of fish in household and local waters, habits of collecting and eating small fish, etc.). For urban areas, it would be relevant to know about the chlorination of water, as fish may be sensitive to high chlorine concentrations.

Health infrastructure and services

Describe briefly the existing health infrastructure in the proposed study area, health care facilities in the non-governmental sector, the availability of healthcare workers, the capabilities of staff, malaria surveillance mechanisms, malaria diagnosis facilities and information, education and communication mechanisms. Mention if there is a larvivorous fish programme and whether related infrastructure and staff exist.

Meteorological information

Collect data on rainfall, humidity and temperature for the study area and relate these to the epidemiological data by month or season to give an indication of variations in the intensity of malaria transmission at different times of the year. The minimum temperature range may be important in deciding on the suitability of some fish species, e.g. *Gambusia affinis* can tolerate very low temperatures, but guppies and other tropical fish cannot.

4. Comparison groups and randomization procedure

The intervention should be allocated at the level of communities rather than individuals. Ideally, the community should be designated as a "transmission zone" for malaria. In many rural settings, this will mean that the village constitutes the community. To avoid the infiltration of vectors from adjacent communities, the communities should preferably be separated by at least 3 km. In certain scattered populations where households are widely dispersed, villages may not exist, but it will often be possible to define separate households, or groups of households, from neighbouring groups.

To estimate the impact of the intervention of the fish, it is necessary to follow a comparison population in parallel in which no intervention or a routine intervention (i.e., no ongoing control measures) is made. Data based solely on changes in morbidity after fish introduction are inadequate because of the year to year variations in malaria transmission. Several intervention communities and several comparison communities will be needed. It is important that no additional intervention is made in the comparison population that could have any effect on the end-points being measured. In order to obtain unbiased results, after the identification of communities (villages/hamlets), randomly allocate the communities to either the intervention (with fish) or the control (without fish) group. For 20 or more communities, simple unrestricted randomization, in which each community is given an equal chance of allocation to either group, is likely to achieve close comparability between the two groups. Alternatively, the comparability of the groups may be improved by matched-pair randomization. The communities are formed into pairs that are expected from baseline data to have a similar level of malaria morbidity. Within each pair, one community is selected randomly to receive intervention. When the number of communities is small, allocation of the communities is done by matched-pair randomization. The improvement in precision achieved by matching depends on how well the baseline information predicts the variations in morbidity during the follow-up that would occur in the absence of an intervention.

The unit of randomization is likely to be the community in the studies envisaged. It is important to stress that several interventions and several control communities will be needed. Taking parasite rates based on a cross-sectional survey in the proposed study area or annual parasite index (malaria cases/1000 population) as a measure of malaria incidence, an expected reduction of 20% to 30% at the end of a 2-year trial and >90% power (the study size should be sufficient to give a high power, i.e. probability of rejecting the null hypothesis, if the reduction in morbidity is large enough to be of substantial public health importance), the sample size should be calculated. Depending on the endemicity of malaria, the protocol should define in which age-group the effect on morbidity is to be assessed, for example children aged 6–11 months, 1–4 years, 5–9 years, 10–14 years and adults.

5. Baseline studies and other preparatory work

It is important to conduct a number of baseline studies and to do other preparatory work prior to the design and performance of the intervention study. The following preparatory work/baseline studies need to be undertaken.

Population census

A population census of the communities within the study area is needed at the start of the study.

Geographical reconnaissance of larval habitats

After the intervention villages or communities are chosen based on randomization, the next step is to carry out geographical reconnaissance to assess the existing number, size, location and types of the mosquito breeding habitats.

Training of staff

It may be necessary to impart training to field and other staff on fish biology, breeding, transportation, fish introduction into new habitats, precautions during handling of fish, fish density determination, entomological and parasitological monitoring and data recording.

Fish stocks

Decentralized fish stocks need to be maintained for initial fish introduction and further replenishments. The size and location of these decentralized facilities will depend on the size of the study area and the availability of locations for such sites.

Baseline malaria parasite rates

It is essential to conduct cross-sectional surveys quarterly (or at least both before and after the main malaria transmission period) each year to determine any periodic/seasonal changes in the parasite rates within the intervention and control communities.

Entomological parameters

A baseline study of entomological parameters covering one year would be most useful, as it would cover possible seasonal variations. Fish work only against the larval/pupal stages, therefore, impact on larval and pupal counts in various sentinel habitats is more easily monitored. Larval counts are made using a standard dipper (300 ml, 9 cm diameter). In large-scale trials covering many villages/communities, at least 30 locations of each type of major habitat should be chosen for larval density monitoring. One can decide on the number of dips to be taken from each habitat according to the size of the habitat (5–20 dips each time). Larval counts should be made weekly and standard forms to record the data should be developed (noting initial details such as type of the habitat, location, size, water depth, presence of aquatic vegetation and visual turbidity/pollution).

The density of adult mosquitoes should be monitored, preferably weekly, by standard techniques (pyrethrum collection, human landing catches, light trap collection) whose details are available in standard entomology texts. Standard forms to record the data should be developed. For pyrethrum collection, four fixed rooms may be chosen randomly from productive houses (based on a few prior collections) in each community/village and collection should be made from a sufficient number of villages such that after randomisation, at least 5–8 villages/communities each are chosen for both the intervention and control groups.

Community and informed consent

It is suggested that the consent of the community leaders or prime movers in the community be obtained before the launch of a study to ensure community acceptance of the use of fish, especially if these are to be released in household storage waters. Mention in the protocol how this would be achieved. Informed consent of individual volunteers who may be exposed to a study process (e.g. mosquito landing catches) should be obtained. A form on informed consent should be developed in the local language.

Meteorological data

Data on rainfall, temperature and humidity should be obtained from the nearest available source.

6. Conduct of the trial and outcome measures

Introduction of fish in larval habitats and their replenishment

Fish are generally introduced at 5–10 individuals per square metre of surface area. (In the Somalia study, *Oreochromis* was applied at the rate of 1 fish/3 m^2 water surface in cemented reservoirs.) These need to be monitored periodically for their continued presence, therefore mention should be made of the mechanism and periodicity of checking and replenishment. Record the data on the introduction and replenishment of fish.

The following outcomes need to be measured.

a) Impact on larval counts

Following the procedure described earlier, larval counts should be made weekly in habitats with fish and in those without fish (in control areas) during the intervention period. Percentage reduction in larval densities should be calculated as described earlier.

b) Impact on adult mosquito population
The density of adult mosquitoes should be monitored from fixed collection sites, as described earlier, during the intervention phase from the villages/communities both in the intervention and in the control areas. Data on densities per room may be transformed to log (+1) values to calculate geometric mean densities for each month. These may then be compared for the two groups of areas and statistical differences may be analysed, say by t-test (if the data are normally distributed), or by the Kruskal-Wallis non-parametric test (EpiInfo software has this facility).

c) Impact on malaria morbidity
Cross-sectional mass blood surveys should be carried out quarterly during the intervention phase. Note: In countries where malaria has been eliminated, such a survey may be used to ensure that malaria is not re-introduced.

Operational sustainability of the use of fish

a) Acceptability, advocacy and information, education and communication needs
Depending upon the study objectives, it may be useful to collect information to determine community acceptance of fish, associated benefits of the intervention tested and advocacy/health education needs. Structured questionnaire-based surveys and qualitative assessments by focus group discussions may be used to develop these ends.

b) Infrastructure development and mechanism
Long-term sustainability of a larvivorous fish programme requires that fish resources be developed locally by involving, for example, fisheries extension services and mechanisms for the transportation and distribution of fish stocks (provision of transport, tools/containers/materials, staff, decentralised stocking tanks, etc.). Identify and mention the prospective agencies that could possibly become partners to sustain the interventions.

7. Cost-effectiveness analysis

The long-term sustainability of the operational use of fish is best determined by a cost-effectiveness analysis with the assistance of a socioeconomist. Whereas analysis of costs and monetary gains of a fish programme (namely of mass breeding and distribution of fish, evaluation of impact and income generated, if any) is more feasible, estimation of the effectiveness of the use of fish vis-à-vis the final objective(s) of the project might become difficult when the fish are used in combination with other methods in an integrated disease control programme. Analysis using cost/benefit ratio is a difficult proposition.

8. Data processing

An operational trial will generate substantial quantities of data. Particular attention must be paid to data processing and equipment needs. Where possible the data should be processed and basic analyses done on site to check that the data collected are of good quality. Computing requirements and the exact data processing system adopted will vary from one study to another, but these should be considered at the study design stage so that adequate provision is made for the computing and statistical requirements.

9. Duration of the study

The duration of the study to test the efficiency of the fish may normally require two to three years, which may include one year of baseline studies and one-to-two years of intervention. (The impact caused by fish may be relatively slow compared with insecticides).

For further information:
Roll Back Malaria, World Health Organization, Regional Office for the Eastern Mediterranean
P.O. Box 7608, Nasr City, Cairo 11371, Egypt
Tel.: (202) 670 5000, Fax: (202) 670 2492/4
E-mail: rbm@emro.who.int
www.emro.who.int/rbm