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Review Article

Review of the bioenvironmental methods for malaria control with special reference to the use of larvivorous fishes and composite fish culture in central Gujarat, India

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ABSTRACT

Mosquito control with the use of insecticides is faced with the challenges of insecticide resistance in disease vectors, community refusal, their high cost, operational difficulties, and environmental concern. In view of this, integrated vector control strategies with the use of larvivorous fishes such as Guppy (*Poecilia reticulata*) and *Gambusia* (*G. affinis*) as biological control agents were used in controlling mosquito breeding in different types of breeding places such as intradomestic containers, various types of wells, rice-fields, pools, ponds and elsewhere in malaria prone rural areas of central Gujarat. Attempts were also made to demonstrate composite fish culture in unused abandoned village ponds by culturing Guppy along with the food fishes such as Rohu (*Labeo rohita*), Catla (*Catla catla*) and Mrigal (*Cirrhinus mrigala*). Income generated from these ponds through sale of fishes was utilized for mosquito control and village development. The technology was later adopted by the villagers themselves and food fish culture was practised in 23 ponds which generated an income of ₹ 1,02,50,992 between 1985 and 2008. The number of villages increased from 13 to 23 in 2008 and there was also gradual increase of income from ₹ 3,66,245 in 1985–90 to ₹ 55,06,127 in 2002–08 block. It is concluded that larvivorous fishes can be useful tool in controlling mosquito breeding in certain situations and their use along with composite fish culture may also generate income to make the programme self-sustainable.

Key words Composite fish culture, larvivorous fishes, malaria, mosquito larval control

INTRODUCTION

Mosquito control through use of insecticides is beset with many inherent problems including their high cost, environmental concerns and more importantly the development of resistance in vector mosquitoes, hence alternative approaches through integrative methods have been attempted in the recent past[1]. Integrated Vector Management (IVM) is described as a rational decision making process for optimal use of resources for vector control and includes methods based on knowledge of local vector biology, disease transmission, utilization of range of interventions often in combination and synergistically as well as collaboration with health sector and other public and private sectors by involving local communities and stakeholders[2]. This is the technology of controlling malaria and mosquitoes through incorporating all those methods which are ecologically safe, cost-effective, long-lasting and suitable to the local conditions.

Due to widespread outbreak of malaria in many of the villages of Kheda district in central Gujarat, where commonly used insecticides were showing resistance, attempts were made to control malaria through non-insecticidal methods based on minor engineering procedures, drainage cleaning, environmental improvement, tree plantations on leveled land, inter-sectoral coordination, promoting information, education and communication and community participation. Larvivorous fishes like Guppy and *Gambusia* were introduced on large-scale and expanded polystyrene (EPS) beads were used in abandoned wells. To generate income and make the programme self-sustainable, schemes like social forestry and composite fish culture were also attempted and environment friendly technologies like improved *chulhas* (smokeless stoves) and solar cookers were added to make this programme towards a holistic rural development programme along with mosquito control[1,3,4]. This feasibility-cum-demonstration study was attempted in high malaria endemic rural areas of Kheda district in central Gujarat during 1980–1990s and some components of the strategy such as food fish culture were later adopted by the villagers and they continued to do work of their own till

2008 when the information was last recorded. The overall interventions of non-insecticidal vector control measures, their impact on mosquitoes and malaria control and thereafter adoption to other ecotypes with different epidemiological situations/settings are described below:

KHEDA PROJECT

Bioenvironmental control of malaria

An alternative integrated disease vector control demonstration-cum-feasibility study based on non-insecticidal methods of mosquito control was started in the rural areas of Kheda district in central Gujarat in the year 1983 following a serious outbreak of malaria in Village Bamroli and other adjoining villages causing severe morbidity and mortality with Annual Parasite Incidence (API) 28 in Kheda and API-51 in Nadiad, the worst affected taluka. As the area is known for insecticide resistance it was thought opportune to start working on the bioenvironmental control of malaria by using simple techniques such as source reduction, minor engineering methods, environmental management, health education, community participation, biological control measures and soliciting inter-departmental coordination. Emphasis was also given on income-generating schemes such as social forestry and food fish culture for rural development and sustaining the community involvement in mosquito control activities.

Study area

Kheda district (now split into Kheda and Anand districts) in central Gujarat in western part of India is situated between 22° 7′ and 23° 18′ North latitude and 72° 15′ and 73° 37′ East longitude with a total area of 7194 km^2. Geographically, the district is plain with some hilly tract in Balasinor and Kapadvanj talukas. There are two perennial rivers, viz Mahisagar and Sabarmati in the district whereas Rivers Vatrak, Shedi, Meshwo and Mohar are seasonal. Except occasional showers in winter, the rainfall is received during June and October (Average rainfall 600–1200 mm). Average mean temperature of 20°C prevails throughout the year and relative humidity (RH) of ≥60% was observed from June to October which is suitable for sporogony. The district has good canal irrigation and out of 10 talukas, seven are under extensive irrigation network through Mahi Kadana Irrigation Scheme. Villages are well-connected by roads with agriculture based economy. The main crops are millet, rice, wheat, cotton, tobacco and ground nut.

Intervention measures

Study on various aspects of bioenvironmental control of malaria was started in 1983. In the beginning, seven malaria prone villages with a population of ~26,000 were selected for the experiments and were designated as complex A. The study was expanded to 14 more villages in 1984 with a total population of ~35,000 termed as complex B, and in 1986, the entire Nadiad taluka covering 100 villages with population of ~3,50,000 was covered under the bioenvironmental strategy. In 1987–88, other adjoining taluka Kapadwanj with 161 villages having a population of ~3,50,000 was also included for the experimental area. To monitor the impact of intervention activities few control villages were also selected in adjoining talukas. Village *Panchayats* (Administrative body) were the nodal points and village heads (*Sarpanch*) were the nodal persons for execution of the work and were briefed about the Malaria Research Centre (MRC), now renamed as National Institute of Malaria Research (NIMR) proposed activities in the target villages. Communities agreed to extend the support and even helped in arranging the tools, manpower and sometimes contributed money for carrying out the mosquito control (source reduction) activities. Primary Health Centres (PHCs), Community Health Centres (CHCs) and Sub-centres were the focal points for collection of baseline data and Medical Officers supported the execution of the intervention work highlighting the crucial role of health systems.

All the villages before starting of the interventions were surveyed for different anthropometric and malariametric parameters such as population and mapping of the village, number of houses, family size, presence of mosquito breeding places such as ponds, pools, wells, rivers, canals, rice-fields, seepage water collection, intradomestic containers, etc and pasting of cards on every house with some basic information giving name of the head of the family and family size. For fever survey, surveillance workers from the same villages were appointed and imparted training to handle malaria cases. Door-to-door surveillance was done every week, slides were prepared from the fever cases, sent to the laboratory for examination and radical treatment was ensured within 48 h. Mosquito collections were made on weekly basis and vector and adult mosquito densities were noted on pre-structured proformae. Similarly, intradomestic and well surveys were done weekly and larval densities were recorded. Immatures were collected and brought to the laboratory for rearing and adult emergence for species-identification and to find out the species-specific breeding sources.

For the control of mosquito breeding, intervention measures were applied, and every week teams of 4–5 daily wage workers with a supervisory staff were deputed in each village and larval control activities were carried out. Major intervention activities performed were the source reduction, i.e. elimination of temporary ditches, pools, and open breeding places, repairing of leakages to prevent waterlogging, cleaning of margins of the ponds, removal of debris, vegetation, and undesired items with the help of larval nets and introduction of larvivorous fishes such as Guppy (*Poecilia reticulata*), *Gambusia (G. affinis)* and *Aplocheilus* (*A. panchax*). Later, mainly Guppy fishes were introduced on large-scale as they were available in abundance. Domestic breeding was controlled through changing of water in containers such as mud pots, cisterns, drums, barrels, etc every week and fishes were introduced in tanks such as overhead tanks (OHTs), under ground tanks (UGTs), inside tanks (ISTs) and other large cemented tanks. To create awareness among the villagers, health camps, live demonstrations, door-to-door visits, video shows and group meetings were arranged and even the villagers and their heads were allowed to visit to the MRC laboratories to get the basic knowledge. Breeding in wells was controlled through the application of EPS beads (@ of 500 g to 1 kg/m^2) in unused wells and introduction of larvivorous fishes in the used wells. Entomological investigations also included the study of anopheline fauna, outdoor resting behaviour of anophelines, species-specific breeding sources and all night mosquito collections to understand the biting rhythms and feeding behaviour of the anophelines.

On the request from the state authorities sometimes mass blood surveys and outbreak investigations were also carried out in other areas as and when there was an unusual high incidence of malaria. Fish fauna of the study area was studied and two surveys were undertaken in the year 1985–86 and in 1991 to know the types of fishes present in the study area, their choice for the habitats and to check their larvivorous potential in laboratory and under field conditions. For mass culture of Guppies, village hatcheries (multiplication ponds) were encouraged. Among the income generating schemes food fish culture and social forestry schemes were also promoted. Inter-departmental coordination was maintained to develop linkages with other departments such as Department of Fisheries, Public Works Department (PWD), Irrigation Department, Forest Department, etc which helped in mosquito control activities. In other collateral activities, smokeless *chulhas* and solar cookers were also demonstrated and massive tree plantation work was taken up in the study villages with the help of Gujarat Energy Development Agency (GEDA), Vadodara and National Wasteland Development Board, New Delhi for over all rural development along with malaria control.

Fish collection and transportation

Fishes were collected from ponds, stocked in large cemented tanks and hatcheries; and transported to desired destination in the following manner: (i) through jeep and trailor with galvanized container and plastic liner for short distance transportation in local areas; (ii) fishes were collected by fine mesh seine nets of sizes 2×5 m, 2×10 m or nylon nets or through hapas (1×1.5×1.5 m) or bamboo nets depending upon the size of the water habitats; (iii) plastic containers such as plastic buckets (200–250 L) were also used or plastic sieve of different sizes were utilized as and when required; (iv) for long distance transportation oxygen cylinders were also used and oxygen was filled in polythene bags; and (v) polythene bags of different sizes were filled with oxygen, tied at the top and kept in tins properly for transportation. Numbers of fishes were kept to around 250 fishes/per bag to avoid overcrowding and to prevent them from suffocation[5].

Impact of interventions on mosquitoes and malaria control

Intervention activities in the entire taluka were in full swing from 1983 to 1989. During the reported period a total of 4,32,775 breeding places were eliminated and to prevent mosquito breeding at the door steps a total of 2730 soakage pits were constructed with the technical support from B.V. Polytechnic, V.V. Nagar, Gujarat at very cheap cost of ₹ 35–60. Temporary breeding places were emptied out through pumps and low depression land and large ditches were filled by the earth work through *Shramdans* by the community. A total of 31,375 tractor trolley loads were used to fill these ditches. Sometimes, this activity was supported by voluntary agencies such as *Nehru Yuvak Kendra,* Youth Clubs, *Mahila Mandal* (women groups), school children, National Service Scheme (NSS) and National Cadet Corps (NCC) cadets, etc and thereafter plantation was done on the leveled land. To prevent mosquito breeding in unused wells EPS beads were applied in 889 wells. These beads form a mechanical layer on the water surface and prevent mosquitoes to lay their eggs and those already present died due to suffocation. This was a cheap and long-lasting solution, as very rarely EPS beads required re-application, when there was disturbance in the layer. These beads were also applied on the slurry (outer open circumference) of the *Gobar* gas plants at some places[6].

To create awareness among the community and enhance their knowledge on vector-borne diseases a total of 172 health camps were arranged and live demonstrations of the malaria parasites, mosquitoes, their larval stages, larvivorous fishes, etc were made. Besides, 1739 group meetings were also organized for science dissemination. School children were also involved in mosquitoes control, and many of the health camps were arranged in schools only. Video shows on mosquitoes and malaria control activities were regular part of the control interventions and a total of 160 video shows were held and documentaries on methods of malaria control were shown. In health education campaigns, posters for exhibitions with the help from Indian Space Research Organization (ISRO), Ahmedabad were made and many exhibitions were arranged on special occasions. Pamphlets, brochures, slogans etc were also prepared and used as and when required. Many of the programmes were telecast on Doordarshan (TV), Ahmedabad and media coverage was done in local newspapers. Villages also donated >5 lakh rupees towards mosquito control activities. On marshy, seepage, waste and leveled area after earth work a massive tree plantation work was undertaken and trees like Eucalyptus (*E. hybrid*), Babul (*Acacia nilotica indica*), Gulmohar (*Delonix regia*), Kashid (*Cassia siamea*), Peltroform (*Peltophorum pterocarpum*), Neem (*Azadirachta indica*), etc. were planted. A total of 8,13,222 trees were planted in the entire Nadiad taluka. In addition 24,156 trees in Kapadvanj and 1,93,088 trees in other talukas of Kheda and Panchmahals districts were also planted. Village nurseries were also promoted to minimize transportation as well as to generate income from the saplings raised[7]. Fish production in abandoned, and unutilized village ponds was also demonstrated as an income generating scheme and other developmental activities such as smokeless *chulhas* and solar cookers were also included in the mosquito control activities to solicit people cooperation and sustain their interest towards holistic way of rural development by controlling mosquitoes. Voluntary donations and sale of fishes was held by *Panchayats* (village administrative body) and this money was invested in repairing drainage, making playgrounds and promotion of vector control activities

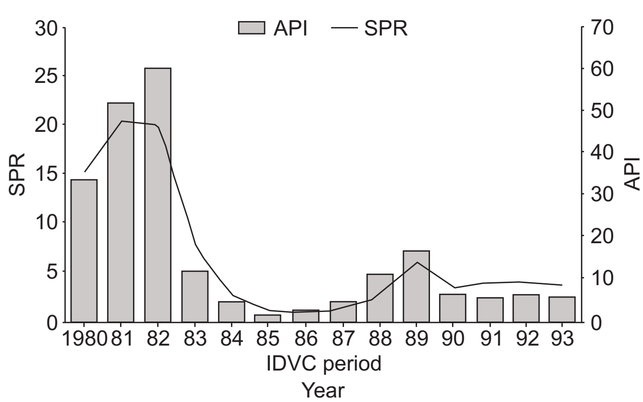

Fig. 1: Impact of control interventions on malaria in Nadiad.

Table 1. A profile of Kheda project: Highlights of the activities (1984–88)

Source reduction	
Breeding places eliminated	4,32,775
Soakage pits constructed	2730
Earth work done (Tractor trolley loads)	31,375
Wells treated with EPS beads	889
Health education and community participation	
Health camps organized	172
Group meetings held	1739
Video shows arranged	160
Money spent by the villagers (in ₹)	5,87,353
Voluntary labour camps (*Shramdans*)	36
Biological control	
Larvivorus fish introduced (in million)	88
Fish hatcheries established	98
Other activities	
Trees planted	10,54,964
Sapling raised in nurseries	11,41,500
Improved *chulhas*	5224
Solar cookers demonstrations	127
Fish production (in ₹)	3,77,860

(Table 1). Results of the entomological findings such as anopheline fauna, species-specific breeding sources, biting rhythms, seasonal prevalence, etc have already been reported earlier[8–14].

Impact of control interventions was visible by decline in number of malaria positive cases from the base period. API/SPR which was >20 in 1981–82 came down sharply and spleen rate also declined (Fig. 1). Mosquito population also reduced considerably and low mosquito and vector densities were recorded in study areas in comparison to control[1]. Intra-domestic positivity was found below 5 in all the experimental villages in comparison to control villages[15] where it was >24. Mosquito breeding in wells was also kept under control and only 6.9% wells were found positive in study areas in comparison to control areas where 29.3% wells were found positive[16]. Intervention and source reduction activities carried out during the period are depicted in Table 1. There was also a wide awareness among the local population which was evident while doing the knowledge, attitude, behaviour and practice (KABP) study.

Role of fishes in malaria control

Fish fauna of the study area

To find out the role of fishes in malaria control, fish fauna of Nadiad taluka was studied and survey of ponds, rivers, canals, pools, seepage water, etc was carried out to collect the fishes. Fishes were captured as described earlier[17,18], counted and identified as per standard methods. Fish fauna survey carried out in experimental villages of Nadiad taluka during 1986–87 revealed 27 types of fishes[19]. A repeat survey was done in the year 1991 to see the change, if any in fishes present in the aquatic ecosystem of the study area and deleterious effect, if any of the large scale introduction of Guppy fishes in various habitats to control mosquito breeding. A total of 35 species were collected from the study area, 21 fishes were common in both the surveys, whereas 9 more species were found in the repeat survey of 1991[17]. However, 5 species could not be recorded in second survey due to their scanty population and one species of the previous survey also recorded second time was identified to species level (*Channa* sp as *Channa striatus*). Most of the fishes were present in perennial ponds, canals, seepage water collections, etc. Among the predatory (weed) fishes *Wallago*, *Chela*, *Notopterus* and *Mystus* were abundant (Table 2). Fish species like *Chela* was predominant in shallow muddy water and found to devour the Guppy population in most instances in case of low water level[17,19].

Screening for larvivoracity and use in vector control

Fishes collected were exposed to laboratory and outdoor test in the field conditions for their larvivorous potential. Out of 27 fishes collected during first survey, 14 fishes were found larvivorous but *P. reticulata* (Guppy) was found abundant, highly efficient and fulfilling the requirements of a good larvivorous fish, hence, chosen for large-scale use in the control programme (Details are

Table 2. Fish fauna of Kheda district

S.No.	Species	Habitats					
		Ponds	Pools	Drains	Seepage water	Canal	River
1.	*Ambassis nama*	+	+	+	+	+	-
2.	*Aplocheilus lineatus*	+	+	+	+	+	+
3.	*Aplocheilus panchax*	-	+	+	+	+	+
4.	*Catla catla*	+	+	-	+	-	-
5.	*Channa punctatus*	+	+	+	+	+	+
6.	*Channa striatus*	-	+	-	+	+	+
7.	*Chela bacaila*	+	+	+	+	+	+
8.	*Chela* species	+	+	-	-	-	-
9.	*Cirrhina latia*	+	-	-	-	-	-
10.	*Cirrhinus mrigala*	-	-	+	+	-	-
11.	*Cirrhinus reba*	+	-	-	-	+	+
12.	*Colisa fasciata*	+	+	+	+	-	-
13.	*Esomus danricus*	+	+	+	+	-	-
14.	*Glossogobius* species	+	+	+	+	+	+
15.	*Heteropneustes fossilis*	-	+	+	+	+	+
16.	*Labeo calbasu*	+	-	-	+	-	-
17.	*Labeo rohita*	-	+	-	+	-	-
18.	*Lepidocephalichthys guntea*	-	-	+	-	-	-
19.	*Mastocembelus armatus*	+	+	+	+	+	-
20.	*Mastocembelus pancalus*	+	-	-	-	+	-
21.	*Mystus punctatus*	+	-	-	-	+	-
22.	*Mystus seenghala*	+	-	+	+	-	-
23.	*Mystus vittatus*	-	-	+	+	+	+
24.	*Notopterus notopterus*	+	+	+	+	-	-
25.	*Ompok bimaculatus*	+	-	-	-	-	-
26.	*Poecilia reticulata*	+	+	+	-	+	+
27.	*Puntius sarana*	+	+	+	+	+	+
28.	*Puntius stigma*	-	+	-	-	-	-
29.	*Puntius sophore*	+	+	-	-	-	-
30.	*Puntius ticto*	+	+	+	+	+	+
31.	*Puntius* species	+	+	+	+	+	+
32.	*Rasbora daniconius*	+	+	+	+	+	+
33.	*Trichogaster fasciata*	+	+	+	+	+	+
34.	*Wallago attu*	+	+	+	+	-	-
35.	*Xenentodon cancila*	+	-	-	-	+	+

(+) denotes presence; (-) denotes absence.

Requirement for an efficient larvivorous fish

- Top (surface) feeder, prolific breeder and small in size, so that they can negotiate for food in shallow water
- Able to breed profusely in confined water
- Able to withstand transport and handling
- Difficult to catch and able to escape their natural enemies
- Worthless or insignificant as food for the human population
- Should not disturb the aquatic ecosystem
- Must be able to withstand temperature variation and reasonable amount of pollution in water

given in Box). Impact of cleaning the margins of the ponds showed that it had considerable impact on the larval density as the fishes were able to reach up to the margins after de-weeding and cleaning. Hence, it was made mandatory to clean the margins of the ponds and pools and removing the grass on the margins to enhance the efficacy of the fishes[19].

Establishment/maintenance of fish hatcheries—Culture, rearing, transportation and supply to other agencies

In experimental villages for mass production of Guppies and their supply to other areas/agencies, village hatcheries were promoted and established near a water source such as wells, tube wells, small ponds, etc. Around 100 hatcheries were maintained/established in experimental villages and guppies were reared and collected from these sites at regular interval and transported to other areas. Before establishing the monoculture of guppies, weed fishes were removed from the water source and their presence and water level was regularly checked. Sometimes fish food or zooplanktons were also added in the habitats.

Establishment of larvivorus fish network for urban areas

Due to emerging threat of malaria and dengue in urban areas Malaria Research Centre (Field Unit), Nadiad in collaboration with Ahmedabad Municipal Corporation took up demonstration project on the management of malaria and dengue vectors in Ahmedabad City during 1999–2000. The most common breeding sites found in the city were cement tanks, ground level tanks, fountains, elevator chambers (lift wells), wells, mill hydrant tanks, cattle troughs and ponds. A larvivorous fish net work was established as a model for urban areas and Guppy fishes were reared and distributed in whole of the municipal limit and there was a sharp decline in malaria cases after the introduction of fishes[5].

Edible fishes (composite fish culture) linked with control of malaria

Each village in the study area (Nadiad taluka) had at least one permanent pond and some seasonal ponds. These ponds were supporting moderate to heavy mosquito breeding and its control was a major concern for the project. Breeding of mosquitoes was mainly confined at the shallow margins of the ponds and in hoof prints, left over pools due to reduction of water at the periphery. Even some ponds were found infested with the water hyacinth (*Eichhornia cressipes*) and these were also supporting mosquito breeding. A survey was carried out to know the mosquito species composition of such ponds and efforts were made to remove the water hyacinth and control the breeding[20,21].

On experimental basis to exploit these ponds, composite fish culture, i.e. mass culture of Guppies along with production of edible fishes was attempted to generate income side-by-side controlling the mosquito breeding. For this purpose in 1985, 8 village ponds were taken from the village *Panchayats* for food fish culture and after removal of weed (predatory) fishes such as *Wallago*, *Channa*, *Notopterus*, *Mystus* and others, these ponds were stocked with the finger lings of Indian major carps *Labeo rohita* (Rohu), *Cirrhinus mrigala* (Mrigal) and *Catla catla* (Catla) as they, occupy column bottom, and surface part of the water respectively and being their different ecological niche there was no competition among them while rearing. However, four ponds had to be abandoned due to large scale poaching and drying due to water scarcity and their fish stock was transferred to remaining ponds. Thereafter in 1986, six ponds were taken and fishes like *Hypothalmichthys molitrix* (silver carp), *Ctenopharyngodon idella* (grass carp) and *Cyprinus carpio* (common carp) were introduced. A total income of ₹ 2,37,840 @ ₹ 9091/ha/yr with production of 1099 kg/ha/yr was generated through the auction of fishes from these ponds. The income generated was utilized for mosquito control activities such as construction of drainage, play ground and other developmental activities[22].

Community involvement in food fish culture as an income generating source for sustainable malaria control

Composite fish culture along with the production of Guppies in some selected ponds of Kheda district, Gujarat generated income for the village *Panchayats* along with the production of guppies for mosquito control and the income generated was utilized for mosquito control and


Collection of fishes from ponds.


Collection of larvivorous fishes from village ponds.


Transportation of larvivorous fishes in jeep with trailor.


Larvivorous fish *P. reticulata* in polythene bags for introduction.


Transportation of fishes in containers with oxygen.


Collection of edible fishes.

other developmental activities of the villages. This created great curiosity, interest and awareness among the villagers as in most villages, ponds were either lying abandoned or not properly utilized. This led to starting of edible fish culture by villagers themselves. Intervention activities were continued from 1983 to 1989 and in 1990 control activities were withdrawn, but villagers continued to do some sort of activities of their own as they were so impressed and motivated by the fish culture activities that they started doing the fish culture of their own. A record of money earned through the auction of fishes from the village ponds was collected from the village *Panchayats* for the period between 1985 and 2008 and data of 5 yr was pooled and depicted in 5 yr blocks for brevity. During 1985 to 2008, in 23 villages food fish culture activities were undertaken. The number of villages increased from 13 in 1985 to 23 in 2008 that generated an income of ₹ 10,250,992 from the fish auction.

Table 3. Income generated from village ponds used for composite fish culture from 1985–2008

Villages/Yr		1985–90	1991–95	1996–2001	2002–08	Total
1.	Davda	0	51500	87000	175827	314327
2.	Dantali	0	0	68751	119106	187857
3.	Dumaral	42076	44650	235000	29607	351333
4.	Tundel	0	0	0	42000	42000
5.	Mitral	29025	37003	169100	134000	369128
6.	Keriavi	18100	51246	47338	164303	280987
7.	Dahegam	0	9000	17500	98769	125269
8.	Vaso	15606	168707	798100	1699960	2682373
9.	Mohrel	0	0	40600	61000	101600
10.	Dabhan	0	0	0	198419	198419
11.	Salun	2000	78205	261889	371827	713921
12.	Kanjoda	48428	65675	104752	166907	385762
13.	Mangharoli	0	10000	36574	21000	67574
14.	Narsanda	1095	167277	282543	242801	693716
15.	Akhdol	4102	9152	36405	29144	78803
16.	Pij	0	0	0	810306	810306
17.	Bamroli	28982	42227	67500	77352	216061
18.	Surasamal	60450	94000	129501	145358	429309
19.	Mahisa	0	0	0	11335	11335
20.	Uttarsanda	39979	211631	644755	482162	1378527
21.	Chalali	0	0	2000	16833	18833
22.	Alina	76402	82037	227002	342804	728245
23.	Kanjari	0	0	0	65307	65307
Total		366245	1122310	3256310	5506127	10250992
No. of villages		13	16	18	23	23

Figures are in Rupees (₹).

There was gradual increase of income from fish auction from ₹ 366,245 in 1985–90 to ₹ 5,506,127 in 2002–08 block (Table 3). The money generated was utilized for developmental and mosquito control activities. This showed the impact of Kheda project on enhancement of knowledge, attitude, behaviour and practices (KABP) of malaria control in rural areas and long-lasting impact of bioenvironmental malaria control strategies which can be replicated in some situations at other places and may help in reducing the over dependence on insecticide-based vector control operations.

Dispersal and colonization

Dispersal of Guppy and *Gambusia* and their colonization in new habitats has been studied extensively in the past[17, 23] and it was found that both the fishes were unable to establish and colonize in new habitats due to the presence of large amount of predatory fishes and lack of regular monitoring. This also reconfirms the fact that these fishes are unable to colonize of their own and need regular supervision if they have to be used in long-term mosquito control programmes. However, it was also established that these do not pose any serious ecological hazard and danger to other fishes of the ecosystem as there was no major change in the fish fauna after their long-term use.

Studies on a local larvivorous fish Aphanius dispar

Aphanius dispar an indigenous delicate larvivorous fish abundantly found in the coastal areas of Gujarat mainly in Kutch was collected and tested in laboratory and under field conditions for mosquito larvivorous potential and showed promising results[24]. Large-scale field trials of *A. dispar* were carried out in Rapar taluka of Kutch district in agricultural field of 4 PHCs during 2005 and 2007. The fishes were released in 19 villages in man made earthen and cemented farm ponds, wells and other permanent water bodies. Significant reduction in the breeding of malaria vectors, *An. culicifacies* and *An. stephensi* was noticed in comparison to control. The fish was found useful in the control of malaria in view of the habit of rainwater harvesting by the inhabitants in north Gujarat (NIMR unpublished data).

Scaling-up use of larvivorous fishes and man power development

In view of the increasing role of fishes in vector-borne disease control and training requirements of the personnel involved in antimalaria programme, scaling-up the use of *Aphanius* or other larvivorous fishes in District Kutch and the State of Gujarat has been carried out. Attempts were also carried out through field demonstrations and training of field staff to strengthen the capacity in larvivorous fish activities as part of overall vector control operations. The long-term objective of the scaling-up use of larvivorous fishes is to reduce the reliance on insecticides and promotion of appropriate and environmental friendly vector control methods and strengthening capacities of personnel involved with vector-borne disease control programme to achieve sustainable vector control based on the principles of integrated vector management (IVM).

Non-insecticidal and fish-based mosquito control interventions at other places

With the landmark discovery of Sir Ronald Ross in 1897 while working in Secunderabad, India in establishing the role of *Anopheles* mosquitoes in transmission of malaria, the mosquito control operations were initiated with emphasis on the environmental and engineering methods of mosquito larval control. There were several examples of successful malaria control such as malaria control in Bombay[25], antimalaria operations in irrigated areas by Clyde during Sarda Canal construction[26], malaria control in Irwin Canal project[27] and Cauvery-Mettur project[28]. Other examples include control of rural malaria transmitted by *An. fluviatilis* by de-weeding[29], growing shade loving plants over tea garden drains to control *An. minimus*[30], etc.

Fishes have been utilized for mosquito control from time-to-time. Around 315 fish species under 7 genera are larvivorous in nature. Exotic fishes like *Poecilia* (*Lebistes*) *reticulata* (Peters 1859), a native fish of South America and commonly known as Guppy was introduced in 1908 and *Gambusia affinis* (Baird & Girard, 1853; common name Top Minnow), a native of Texas and widely distributed in the world was imported from Italy and introduced in India in 1928[18]. Since 1937, fishes have been employed for the control of mosquito larvae and in urban malaria programme[31,32] but with the advent of DDT in 1940s and its large scale use in national malaria control programme along with other potent insecticides brought spectacular success and malaria was almost near eradication and non-insecticidal approaches were altogether forgotten. But euphoria of success short lived and malaria resurged back with vengeance and reached a peak in 1976 with 6.4 million cases and many deaths[33]. In view of the same, alternative approaches were revived to minimize dependence on insecticides and Kheda project demonstrated that controlling malaria through integrated approaches is not only feasible but also cost-effective and environmental friendly[1,3,4,34]. It also brings out semi-permanent to permanent changes and create awareness among the communities. Kheda project became a role model and was replicated in different eco-epidemiological settings and produced satisfactory malaria control and reduced mosquito nuisance[35]. Though fishes have been used on experimental basis during 1970–80s and found useful in control of mosquito breeding in different habitats like wells, pits and tanks (Table 4) but their large-scale use gained momentum during demonstration of Kheda project and based on the successful demonstration on the utility and use of larvivorous fishes in mosquito control in Kheda, Gujarat the fishes were attempted in different eco-epidemiological zones of the country such as BHEL Industrial set-up at Hardwar[36], tribal dominated rural areas of Jabalpur[37] and Shankargarh[38], urban areas of Chennai[39], Ahmedabad[5] and Goa[40] and rural irrigated areas of Shahjahanpur[41-43], Rourkela[44], Haldwani[45] and others[46–53].

Many of the components of the Kheda project were also included in the National Malaria Control Programme and even in Global Malaria Control Programme. The impact of integrated and bioenvironmental approaches to malaria and mosquito control with focus on the use of fishes have been attempted at various places and have shown promising results[31, 54-56]. Utility of larvivorus fishes in mosquito control programme is a known phenomenon and has been used at various places. Ghosh *et al*[57, 58] demonstrated the control of mosquitoes and malaria in Karnataka by the use of fish *P. reticulata.* He also reported that *Poecilia* was effective in closed-ecosystem such as wells, while *Gambusia* in open ecosystem like ponds and streams[59]. Menon & Rajagopalan[60] introduced *G. affinis* and *Aplocheilus blockii* in wells of Pondicherry and found that the later showed high tolerance for salinity and pH. Larvivorous fish network was developed to control urban malaria in Ahmedabad and there was a sharp decline in intradomestic positivity by the introduction of fishes[5].

In some other studies on fishes, Jayasree & Panicker[61] demonstrated that larvivorous potential of some indigenous fishes *Ophiocephalus striatus* and *Macropodus cupanus* showed high efficacy against mansonoides larvae in Sherthallai region of Kerala. Phytophagous fishes, viz. *Ctenopharyngodon idella* and *Ospronemus gouramy* were also used to control mansonoides mosquitoes by checking the growth of aquatic weeds, which support mosquito breeding[62,63]. Larvivorous potential of some cypriniformes fishes and grass carp and major carp was evaluated and composite fish culture was also attempted

Table 4. Larvivorous fishes used for the control of mosquito breeding in India

Place/Area	Fish species	Habitats	Reference
Calcutta (now Kolkata)	Guppy	Surface drains	Hati & Saha[53]
Ghaziabad	*P. reticulata* (Guppy)	Wells	Ansari *et al*[52]
Goa	*A. blockii*	OHTs and other containers	Kumar *et al* [40]
Gujarat (Rural areas)	*P. reticulata* *A. panchax* *G. affinis* *Aphanius dispar*	Wells, Ponds, Rice fields, Domestic containers and Cement tanks	Kant *et al*[16] Gupta *et al*[15] Sharma *et al*[1] Haq & Yadav[24]
Ahmedabad City	Guppy *Gambusia*	Tanks, Fountains, Wells, Hydrants and Ponds (Urban areas)	Haq *et al* [5]
Haldwani	*G. affinis*	Tanks and *Pokhars* (Ditches)	Malhotra & Sharma[45]
Hardwar	Guppy *Gambusia*	Ponds, Drains and UGTs (BHEL Industrial Complex)	Dua & Sharma[36]
Hyderabad	*G. affinis*	Wells	Sitaraman *et al*[50]
Jabalpur	Guppy *Rasbora daniconius*	Wells	Singh *et al*[37]
Karnataka (Silk industry area)	*P. reticulata* *G. affinis*	Wells, Ponds and Streams (Kolar Gold Fields)	Ghosh *et al*[59]
Madras (now Chennai) City	*Gambusia*	OHTs and Wells	Chandrahas & Venkataramnaiah[39]
Pondicherry (now Puducherry)	*G. affinis* *A. blockii*	Wells	Menon & Rajagopalan[60]
	G. affinis	Casuarina pits	Rao *et al*[49]
Pune	*G. affinis*	Field tanks	Dixit *et al*[47]
Rourkela	*Danio rerio* *Oryzias melastigma*	Rice-fields	Yadav & Das[44]
Shankargarh (Allahabad)	*Colisa fasciata*	Wells	Tiwari[38]
Shahjahanpur	*G. affinis*	Ponds, Pools, OHTs and Rice-fields	Das & Prasad[41] Prasad *et al* [42,43]

OHTs—Over head tanks; UGTs—Under ground tanks; A— *Aplocheilus*; G— *Gambusia*; P— *Poecilia.*

in ponds of rural areas of Pondicherry[64]. The cost benefit analysis of fish culture strategy towards control of mansoniodes in Kerala showed usefulness of fishes in vector control[65]. Edible fish *Oreochromis niloticust* (formerly *Tilapia nilotica*) has been used in Kenya to control mosquito immatures and 94% reduction was observed in *An. gambiae* and *An. funestus*, while smaller in size and later larger fishes were harvested as a source of income[66]. Fishes were also used in the control of mosquito immatures in Tanzania[67] and the Gambia as well[68]. It has been shown that mass culture of edible fishes along with the larvivorous fish as an income generating source is an innovative concept and may be useful alternative to insecticide dependant vector control programmes.

Integrated vector management based on the use of larvivorous fishes is an environment friendly, effective, acceptable and self sustainable concept as fish offer advantage of self replicating and do not require repeat application, and their composite culture along with food fishes may also generate the income and make the mosquito control programme self sustainable, however, they may not be applicable in all situations, hence, should be used wherever feasible and may prove effective in bringing the mosquito population down to a significant level which will ultimately help in reducing the disease burden to a significant level and insecticides may be kept for the firefighting situations.

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