

Use of Gambusia Fish in the Malaria Eradication Programme of Iran

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Following the discovery of the role of the *Anopheles* mosquito in transmitting malaria, vast campaigns for the improvement of environmental sanitation and vector control were initiated and the attention of scientists in this field, in North America, was drawn to biological methods of malaria control and to the use of larvivorous fish.

During experiments with different fish, American ichthyologists in the 1920s concentrated on a small fish with larvivorous characteristics, the mosquito fish *Gambusia affinis*. It is a viviparous species, and because of its small body it does not possess any food or economic value.

The origin of gambusia, according to the available information, was in the southern and eastern waters of North America, including Texas, Cuba and Mexico. From there, it has been introduced progressively into Spain, some eastern European countries, Italy and North Africa as a measure of malaria control (Bay, 1967).

As examples of early reports on the effectiveness of gambusia, Hackett (1931) states that the introduction of gambusia in an area of 20.7 km² in Istria, on the Adriatic, reduced the spleen rate from 98% in 1924 to 10% in 1930. Hildebrand, in 1925, reported that fish in Georgia, in the USA, had been able to reduce anopheline larval densities in ponds and swamps on average by 50% and culicine larval densities by 80%.

In Iran, during the years 1922–30 when health activities were being expanded in the country and the northern provinces were rapidly developing, gambusia were brought from Italy and were introduced into the Ghazian marshes on the Caspian littoral. At the same time, drainage of these swamps was started. Malaria, at that time, was hyperendemic in most parts of this area and was a principal factor hampering development. Unfortunately, during the Second World War, these activities were interrupted, but, despite this, gambusia fish remained and reproduced in the marshes around Bandar Pahlavi so that it was possible in 1966 to collect them from this area and to reintroduce them to the south.

Reasons for using gambusia in the malaria eradication programme in Iran

Following the introduction of residual insecticides, larval control measures were given low priority in malaria eradication programmes. However, the technical, operational and administrative difficulties encountered in interrupting the transmission of malaria by residual spraying alone, have again directed the attention of malaria workers to the importance of larval control, including the use of larvivorous fish as a supplementary attack measure.

The main difficulties of the Iran Malaria Eradication Programme, which made necessary the application of combined attack measures, are as follows:

The problem of resistance. In the south of Iran, *Anopheles stephensi* developed resistance to DDT in 1957 and to dieldrin in 1960. To replace these insecticides, malathion has been used for residual spraying.

Exophily and exophagy. Other vectors such as *An. fluviatilis*, in the southern highlands of the Zagross Range of mountains, and *An. superpictus*, in most areas of the Iranian plateau, spend the greater part of their life-cycle in shelters outside human dwellings. Despite several years of residual spraying and the continued susceptibility of these vectors to DDT, transmission still continues in some areas where these vectors are found.

Movement and temporary dwellings. In the mountainous areas, particularly along the Zagross Range, there is considerable movement of population to temporary dwellings (tents, huts, etc.) which are mostly erected after the spraying round. Total coverage by insecticide spraying is not feasible as these shelters are moved from place to place and the residual effectiveness of the insecticide on these sprayed surfaces is brief.

The gambusia fish

The gambusia fish is a viviparous species also called "mosquito fish". It is of the Poeciliidae family, of which there are two known subspecies: *Gambusia affinis holbrooki* and *G. a. affinis*. The

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subspecies occurring in Iran has been identified as *G. a. holbrooki*.¹

Morphology. The main distinguishing features of these fish are the relatively flat head, the small body and the projecting mandibles. Because of these characteristics, the fish is able to catch and eat the surface-feeding mosquito larvae, its small size permitting it to live in shallow pools. The male is smaller than the female, and the latter has two dark spots under the abdomen. The adult male measures from 18.9 mm to 45.4 mm in length, but the female may reach 60 mm.

Biology. The gambusia fish lives in fresh water but adapts reasonably well to different conditions. It grows and reproduces faster in tropical areas, but as evidence of its adaptability, it is found to reproduce under very diverse climatic conditions in different areas of Iran. The original source, in marshes on the Caspian Littoral, has a Mediterranean type of climate. From here, it was transported to the south of Iran, firstly to a semi-tropical area where it readily adapted itself to the new conditions and started breeding. From there, it was introduced to the torrid areas along the Persian Gulf. Thus in Iran, it has been possible to observe the establishment of gambusia in such different environments as the Caspian Littoral, areas with moderate humid climate, the Shiraz and Shahreza areas with dry temperature weather, and Kermanshah and Isfahan, which are dry with cold winters and snow, and the south of Iran where the climate is very hot and humid (Rostami, 1963).

The number of larvae eaten daily by each gambusia depends on the larval density; the female can eat more than the male. Where larvae are plentiful, one fish is able to eat as many as 94 pupae or 104 fourth-stage larvae per day. Naturally in order to be effective, the number of fish should depend on the area of water surface and the density of larvae. In extensive breeding places, about 15 females and 1 male are sufficient for each square metre of water surface. For smaller areas without any vegetation, 2 females and 1 male per square metre are sufficient.

Methods used in Iran for transporting gambusia

Transportation from the original sources to the raising ponds. As already mentioned, the main

source of gambusia in Iran was the marsh of Ghazian on the Caspian Littoral. In 1966, about 10 000 fish were distributed from this marsh to raising ponds in Shiraz, Kerman and Kermanshah. Suitable places for the fish had been selected in advance. The distance from origin to destination was from 1000 km to 2000 km: they were transported on a Jeep pick-up on which an oxygen cylinder was installed. Double-walled polythene bags of 30–40 litres capacity were used to transport the fish over long distances, the bags being kept in strong wooden boxes. About 300 fish were placed in each bag which was half-filled with water, then pumped full with oxygen and sealed. The bags were checked once every 2 hours during transportation and, if they had collapsed, oxygen was added. In this way, very few fish died on the journey and they could be easily introduced into the ponds already prepared. It was found that the mortality was even lower if the fish were not fed for 24 hours before being transported.

Types of raising pond. The raising ponds selected were of two different types: some were artificial (pools or cisterns) and the others were natural. In the artificial ponds the water was of uniform depth, the sides were smooth and there were no algae, thus there was no shelter for the larvae. As the fish find difficulty in feeding in such places, the females were at first put in square open-top boxes of different sizes up to 1.5 m wide, made with a light wooden frame and covered with 3.5-mm galvanized mesh or with standard plastic fly-screening. These boxes were weighted with a stone and were placed half-immersed in the water so that the fish could be fed easily. If larvae were not sufficient as a food source, more larvae or fish meal were added until they started to reproduce. In this manner in a few months the numbers were greatly increased so that it was no longer necessary to isolate the females.

In the natural ponds, which had uneven sides, shallow places and some growth of algae, it was not necessary to separate the females because the young fish could take shelter and avoid being eaten by the bigger fish. Feeding was easier in these places because of abundant aquatic insects and different larvae, and reproduction here was very rapid.

Transportation to Shahrestans. After establishment and satisfactory reproduction in the raising ponds, the fish were transported to the operational areas. As the distances did not involve more than

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3 or 4 hours travelling time, simple means of transportation were used. The containers used were 25-litre PVC cylindrical churns with screw tops in which airholes were made. Four of these churns filled a specially constructed wooden box lined with plastic sponge to minimize jostling the fish when the vehicle was moving. The vessels were half filled with water to which about 300 fish were added and the remaining space was left for air. The driver had to change the water in the plastic vessels approximately every hour, using fresh water which he carried in a spare drum.

Distribution of fish to larval breeding places. In areas where gambusia fish are included in the operational programme, the district leader of the Malaria Eradication Organization (MEO) is made responsible for the distribution of the fish. Sufficient fish are taken from the Shahrestan stocks and are distributed among the pools, swamps, and streams of the district as required. Every district leader has a number of plastic churns and a man to distribute fish to the different larval breeding places. The district leader is responsible for checking the breeding places for larvae and he decides whether fish are required. The presence of gambusia in breeding places is routinely checked at all distribution points at the end of any rainy period during which flooding may have occurred, and also at regular two-monthly intervals. In addition a number of breeding places are routinely checked at fortnightly intervals for entomological purposes and the presence or absence of gambusia is also noted then.

Factors reducing the efficiency of gambusia

Despite the considerable potential of gambusia fish for eating larvae, there are certain natural factors and difficulties which restrict their effectiveness and make complete coverage impossible. Among these factors are the following:

(1) *Gambusia* does not reproduce in shallow wells where the water is regularly disturbed (this has been observed in the shallow wells in Bandar-Abbas which are breeding places of *Anopheles stephensi*);

(2) the fish cannot live long enough to reproduce where there is insufficient light and oxygen, as in cisterns and wells;

(3) rain and floods may wash away the fish;

(4) after the floods, temporary breeding places form in depressions left in river beds and as these pools eventually dry up, gambusia cannot be established in them;

(5) the water level of the larval breeding places is constantly changing because of climatic and natural factors and some places may be left without fish, thus allowing larval breeding;

(6) the anopheline breeding places are not limited to permanent and known wet areas where fish can be introduced;

(7) it is not always possible to use gambusia in house pools or goldfish ponds or waters used for raising edible fish. Also, inhabitants may refuse gambusia on the grounds that they dirty the water.

Observations and discussion

The Iran Malaria Eradication Programme has introduced gambusia as an auxiliary measure along with other attack measures such as residual spraying, detection and treatment of cases, mass drug distribution and the application of larvicides. Since in most areas of the south these measures have been combined, it has not been possible to evaluate on a large scale the effectiveness of any one measure.

In particular, gambusia distribution has not been the only attack measure in an area of reasonable size, so that no firm conclusions can be drawn on the effectiveness of this measure. However, during the two years, 1967 and 1968, of distribution of gambusia, some observations have been made which, in a general sense, confirm the efficiency of this measure.

For instance, no anopheline larvae are now found in the numerous stagnant waters around Kermanshah and Shahabad-Gharb following the introduction of fish to all breeding places. Likewise a considerable decrease of anopheline larvae has been noticed in the breeding places in Fars Province. For example, in a breeding place of 15 000 m² in the village of Bisheh Baba Haji near Shiraz, where gambusia was introduced, no larvae were seen during the period from May to October 1968, despite the high density recorded in the same period in previous years. On the other hand a large number of larvae was captured from a small breeding place nearby where no gambusia had been introduced.

In Bahramabad village near Bushehr, in 2 water collections 3 m apart, larvae were not found in one which contained gambusia, while in the other, where no fish were evident, there were many larvae.

Another good example demonstrating the efficiency of gambusia in destroying larvae is the marsh of Islamloo village near Shiraz. The marsh, 18 km × 12 km, is a large potential larval-breeding place and the surrounding villages had high malaria

prevalence prior to the introduction of gambusia in 1967. The village of Islamloo and the marsh are surrounded by rocky hills which provide favourable shelters for exophilic *Anopheles* species such as *An. superpictus* and *An. fluviatilis*. The vectors in this area are *An. stephensi*, *An. superpictus*, *An. fluviatilis* and *An. d'thali*. In 1967, out of 127 blood slides examined from Islamloo village, 27 positive cases were found (about 20%). This village had been sprayed with DDT in May of that year and this spraying was the only other attack measure used. In routine surveys made in 1968, no larva of any species was collected from this marsh. In 1968, out of 39 slides examined only 2 were positive.

In conclusion, the Iran Malaria Eradication Organization believes that, although the use of gambusia fish should never be employed as the sole antimalarial attack measure, a considerable benefit can result in some areas from the distribution of these fish. The decrease in anopheline density has been quite striking in some regions, up to a point where the contact between man and vector had become very low. This improvement has been achieved at low cost since the maintenance of gambusia incurs very low running expenses compared with the application of chemical larvicides, and costs have in most cases been confined to the initial transportation and distribution expenses together with the hidden costs of periodic checking on the presence of fish and the absence of larvae in potential breeding places. The importance of this checking must be emphasized. It is imperative to instil into those responsible that, once gambusia have been distributed in an area, a regular watch for their continued presence must be maintained. As this paper has pointed out there are many factors that may interfere with the development of the fish, factors such as extreme temperatures, flooding or drying, natural

predators, or changes in the chemical nature of the water.

In Iran, the distribution of gambusia fish has been carried out in over 3000 permanent water collections in the south, and during 1969 over 1½ million fish were distributed. It has proved of particular value where the prevalent vector species have been mainly exophilic and thus little affected by residual spraying. The breeding places of these vectors have often been in areas not suitable for the application of chemical larvicides, in the edges of streams, in areas with standing vegetation or in rocky pools, and biological vector control with gambusia has assumed considerable importance. As the prevalence of malaria in south Iran decreases, exophilic vectors will probably play a more and more important part in transmission and consequently all available forms of attack will be required. It is felt that the use of gambusia fish can help in attaining the goal of eradication of malaria from the whole country.

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