USE OF Gambusia FISH IN THE MALARIA ERADICATION PROGRAMME OF IRAN

by

I. Tabibzadeh, G. Behbehani and R. Nakhai
Malaria Eradication Organization, Ministry of Health, Teheran

1. Introduction

Following the discovery of the role of the Anopheles mosquito in transmitting malaria, vast campaign measures 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 1920's concentrated on a small fish with larvivorous characteristics named Gambusia. It was a viviparous species, and because of its small body did not possess any food or economic value.

The origin of Gambusia, according to the available information, was the southern and eastern waters of North America, Mexico, Texas and Cuba. From there, it had progressively been introduced to Spain, 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, the United States of America, 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 1922-1930 when health activities were being expanded in the country and the northern provinces were rapidly developing, Gambusia fish were brought from Italy and were introduced into the Ghanian 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 for introduction to the south.

2. Reasons for using Gambusia fish 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 supplementary attack measures.

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.
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 A. fluviatilis, in the southern highlands of the Zagross Range, and A. 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.

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

Morphology

The main distinguishing features of these fish are a relatively flat head, a small body and projecting mandibles. Due to these characteristics, the fish is able to catch and eat the surface feeding mosquito larvae, its small body permitting it to live in shallow water. The male is smaller than the female, which has two dark spots under the abdomen. The adult male measures from 18.9 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).

1 By Luis R. Rivas, Fishery Biologist of the Fish and Wildlife Service, United States Department of the Interior through the courtesy of Thomas D. Mulhern, Senior Vector Control Specialist, Department of Public Health, State of California, the United States of America.
The life cycle, growth and reproduction of Gambusia have not been studied in the laboratory by the Iran Malaria Eradication Organization. However, from field observations, reports from the Iran Fisheries and other sources, the female, in favourable weather (over 15°C), matures and is able to reproduce within a period of two months. In areas where the summer is short, reproduction may not occur until the age of eight to 10 months, after the passage of a winter. About 17 days after fertilization, the female will start to deliver live young fish and will continue delivery over a period of approximately one month. About 300 young are usually produced at each brood but numbers as high as 428 have been reported. The breeding season in temperate climates lasts from May to September and in warmer climates from April to November. A single fertilization is sufficient for two to three broods and each female is able to have about four broods during its life span. At birth, the young fish is 1.4 mm thick and 2.1-8.1 mm long.

Gambusia can live under different temperatures. If the water is deep enough, Gambusia is able to survive summer air temperatures of 45°C, as well as winter ones of several degrees below zero.

The raising of Gambusia fish

The following points should be considered for the establishment, culture and rapid reproduction of the fish in new areas:

change of living conditions should take place gradually;

the quantity of salt in the raising ponds should not exceed 20 g per litre. Although the fish may survive in water of salinity up to 52 g per litre, it cannot reproduce or live long at this salinity;

the raising ponds should not be subjected to strong water currents, and especially should be protected from heavy rain and floods;

adequate light is necessary so that wells and cisterns do not make suitable places for reproduction nor should the ponds be covered extensively with algae growth;

the fish should be artificially fed if there are not enough larvae or other natural foods in the raising place;

chlorination of the water beyond the tolerance margin or the presence of insecticides will kill many of the fish;

the surface area of the artificial ponds used have varied widely, generally from 10 to 12 m long by 3-5 m wide, but the important factor is the depth. In areas subjected to large differences in summer and winter temperatures, the depth should be at least 1.5 m to permit the fish to seek protection from extremes of heat and cold.

Although some sources have recommended that Gambusia in raising ponds should be protected from the numerous natural enemies, this has not been found of importance in Iran; nor has the ratio of males to females, as the number of fish supplied to any breeding place has been much greater than the number necessary to establish reproduction.

Efficiency of Gambusia

Gambusia is able to eat most forms of aquatic life, such as molluscs, water insects, and different anopheline and culicine larvae. Moreover, it also eats the eggs and young of other fresh-water fish and thus may reduce indigenous fish populations. Young Gambusia are also sometimes eaten by adult fish, but in general they have a preference for mosquito larvae.
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 much as 94 pupae or 104, fourth stage larvae per day. Naturally in order to be effective, the number of fish should depend on the water surface and the density of larvae. In extensive breeding places, about 15 females and one male are sufficient for each square metre of water surface. In smaller water collections without any vegetation, two females and one male per m² will be enough.

4. 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 had been selected in advance for the fish. The distance from origin to destination was from 1000 to 2000 km. The means of transport used was a Jeep pickup on which an oxygen cylinder was installed. Double-walled polythene bags of 30-40 litres capacity (Fig. 1) were used during the transporting of the fish over long distances, the bags being kept in strong wooden boxes (Fig. 2). About 300 fish were placed in each bag which were half filled with water, then pumped full with oxygen and sealed (Figs. 3a and 3b). The bags were checked once every two hours during transportation and, if collapsed, oxygen was added. In this way, the fish reached their destination with a low mortality and could be easily introduced into the already prepared ponds. It was found that the mortality is even lower if the fish are 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 other natural water collections. In the artificial ponds the water is of uniform depth, the sides are smooth and there are no algae, hence there is no shelter for the larvae. As the fish find difficulty in feeding in these places, the females were initially put in square open top boxes of varying size up to 1.5 m wide, made with a lightwood frame and covered with 3.5 mm galvanized mesh or with standard plastic flyscreening. These boxes were weighed with a stone and were placed half immersed in the water so as to be accessible for the feeding of the fish. If larvae as a food source were not sufficient, more larvae were added or the fish were fed with powdered fish meal 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, with uneven sides, algae growth and shallow places, it was not found 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 areas of operations. Simple means were used for transportation as the distances did not involve more than three or four hours travelling time. The containers used were 25 little PVC cylindrical churns with screw tops in which airholes were made (Fig. 1). Four of these churns filled specially constructed wooden boxes lined with plastic sponge to minimize jostling the fish when the vehicle was moving (Fig. 2). The vessels were half filled with water to which about 300 fish were added and the remaining space was left for air (Fig. 3c). 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 is 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 Shahrrestan stocks and put in one of the water collections at the district level. This water collection may be a pool, a swamp, standing water, a slow stream, a spring, or other source. Every District Leader has a number of plastic churns and a man to distribute fish in the different larval breeding places. He may be helped by the driver of the MEO vehicle which is available at the district level. The District Leader, while supervising his men and carrying out his routine duties, will check breeding places for larvae and should they require fish he will notify the man responsible for putting fish in them. Normally, the District Leader carries with him a number of fish in a plastic vessel to use wherever needed. The presence of Gambusia in breeding places is routinely checked in 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 noted then too. If, through natural causes, the fish in them have been lost or if the breeding place needs replenishing, more fish are supplied.

5. Factors reducing the efficiency of Gambusia

Despite the considerable larvivorous potential of Gambusia, there are certain natural factors and difficulties which restrict complete coverage and full effectiveness of Gambusia. Some of them are:

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

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

rain and floods may wash away the Gambusia;

after the floods, temporary breeding places form in depressions left in river beds. As these pools eventually dry up, Gambusia cannot be established in them;

the water level of the larval breeding places changes constantly because of climatic and natural effects. The decrease of water level may leave some pits without fish, thus allowing larval breeding;

the anopheline breeding places are not limited to permanent and known water collections where fish can be introduced;

it is not always possible to use Gambusia in house pools or gold-fish ponds or waters used for raising edible fish. Also, inhabitants may refuse Gambusia on the grounds that they dirty the water.

6. Observations and discussion

The Iran Malaria Eradication Programme has used Gambusia fish as an auxiliary measure along with other attack measures such as residual spraying, detection and treatment of cases, mass drug distribution and larviciding. 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, in no area of any size has Gambusia distribution been the only attack measure, 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-Charb following the introduction of fish to all larva-breeding places. Likewise a considerable decrease of anopheline larvae has also been noticed in the breeding places in Ostan Fars. For example, in a breeding place of 15 000 m² in the village of Bisheh Baba Haji of Gharabagh Dehistan of 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 of Shabankareh Dehistan of Bushehr Shahrestan, in two water collections three metres 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 may be given by the marsh of Islamlou village in Gharabagh Dehistan of Shiraz. The marsh, 18 x 12 km, is a large potential larval breeding place and the surrounding villages had high malaria incidence prior to the introduction of Gambusia in 1967. The village of Islamlou and the marsh are surrounded by rocky hills which provide favourable shelters for exophilic Anopheles species such as A. superpictus and A. fluviatilis. The vectors in this area are A. stephensi, A. superpictus, A. fluviatilis and A. d’thali. In routine surveys made in 1968, no larva of any species was collected from this marsh. In 1967, out of 127 blood slides examined from Islamlou 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 1968, out of 39 slides examined only two 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 requires virtually no running expenditure compared with chemical larviciding, 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 Gambusia 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 which may interfere with the development of the fish, factors such as inclement temperatures, flooding or drying, natural predators, or changes in the chemical nature of the water.

In Iran, Gambusia distribution has been carried out in over 3000 permanent water collections in the south and during 1969, over one and a half 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 chemical larviciding, 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 malaria incidence in south Iran decreases, exophilic vectors will probably play a more and more important part in the continuing of 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.
Il y a quelque cinquante ans, des poissons larvaires du genre *Gambusia* avaient été importés d'Italie et introduits en Iran pour servir à la lutte antipaludique dans les marais de Ghazian, au bord de la mer Caspienne.

Récemment, pour renforcer les mesures d'éradication du paludisme en Iran, ces poissons - pour être précis *Gambusia affinis holbrooki* - ont été utilisés à grande échelle dans le sud du pays où la résistance du vecteur et son exophilie posent des problèmes. On a constaté qu'il était facile d'implanter l'espèce dans différentes régions d'Iran aux caractéristiques climatiques très diverses, pourvu que l'on maintienne des conditions appropriées dans les bassins d'élevage et que l'on dispose de moyens adéquats pour transporter les poissons vivants sur des centaines de kilomètres. L'article donne des précisions sur les précautions à prendre.

De l'avis de l'organisme dont relève l'éradication du paludisme en Iran, l'utilisation des poissons *Gambusia* ne doit pas être la seule mesure d'attaque contre le paludisme, mais elle permet d'obtenir d'excellents résultats dans certaines zones. On a enregistré dans plusieurs régions une diminution frappante de la densité des anophèles, au point que les contacts entre l'homme et le vecteur sont devenus très rares. Cette amélioration a été obtenue à peu de frais, car l'entretien de population de *Gambusia* représente une dépense infime par rapport au coût des applications de larvicides chimiques; dans la plupart des cas, seuls le transport initial et la répartition des poissons ont entraîné quelques frais. D'importantes opérations de contrôle sont toutefois à assurer. Il est indispensable de faire comprendre aux agents responsables que, lorsque des poissons *Gambusia* ont été introduits dans une zone, il faut vérifier périodiquement qu'ils s'y maintiennent. De nombreux facteurs peuvent en effet entraver leur développement, notamment les températures inclémentes, les inondations ou la sécheresse, la présence de prédateurs naturels ou des changements dans les caractéristiques chimiques de l'eau.

Plus de 3000 rivières et étendues d'eau du sud de l'Iran ont été empoisonnées avec *Gambusia*; au cours de la seule année 1969, plus d'un million et demi de poissons ont été lâchés. Cette méthode de lutte antilarvaire s'est révélée particulièrement utile là où la principale espèce vectrice est essentiellement exophile et donc peu affectée par les pulvérisations à effet rémanent. Les gîtes larvaires des vecteurs étant souvent des endroits qui ne se prêtent pas à l'application de larvicides chimiques (bords des rivières, zones à végétation permanente ou cuvettes rocheuses), la lutte biologique à l'aide de *Gambusia* a pris une importance considérable. Au fur et à mesure que l'incidence du paludisme diminuera dans le sud de l'Iran, les vecteurs exophiles jouerront probablement un rôle de plus en plus important dans la persistance de la transmission et il faudra alors faire appel à toutes les méthodes d'attaque possibles. Il semble que l'utilisation des poissons *Gambusia* soit un des moyens qui permettront de parvenir à l'éradication du paludisme sur l'ensemble du territoire.
ACKNOWLEDGEMENTS

This paper has been compiled from many sources by members of the staff of the Iran Malaria Eradication Organization. Mr M. Hoorfar, Field Operations Chief, Ostan Guilan, has been among those in the field who have been closely connected with the project of propagation of Gambusia. However, it is also largely due to the many other field workers, too numerous to mention here by name, that it has been possible to prepare this paper. We would also wish to express our thanks for the co-operation rendered by the authorities of the Iran Fisheries Company.

REFERENCES


Hackett, L. W. (1931) Recent developments in the control of malaria in Italy, Jour. S. Med. Assoc., 24, 426


Rostami, I. (1963) Malaria control in Iran - Publication of Ahwaz Agricultural College, No. 5
FIGURE 3A. PLACING FISH IN HALF FILLED DOUBLE-WALLED POLYTHENE BAG
FIGURE 3B. POLYTHENE BAG BEING FILLED WITH OXYGEN
FIGURE 3C. TYPE OF PLASTIC CONTAINERS USED (NOTE OXYGEN CYLINDER ON VEHICLE)