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Geographical distribution and evaluation of mosquito larvivorous potential of *Aphanius dispar* (Rüppell), a native fish of Gujarat, India

Sarfarazul Haq[1] & Rajpal S. Yadav[2]

[1]*National Institute of Malaria Research (ICMR) Field Unit, Nadiad, Gujarat, India;* [2]*Vector Ecology and Management, Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland*

ABSTRACT

Background & objectives: Certain indigenous fish can play potential role in vector control. The study recorded distribution of *Aphanius dispar* (Rüppell) in its native habitats in Gujarat, India and evaluated its larval propensity for Indian mosquito vectors.

Methods: Fishes were surveyed in various districts of Gujarat and samples were collected from coastal habitats and were identified to species. Physicochemical properties of water samples were analysed in the laboratory. Five laboratory acclimatized adult fish (2.2–3 cm) released in chlorine-free water in glass jars were provided with 500 larvae daily. Five replicates were run. Daily consumption of I to IV instars of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* was recorded separately for three days. *Anopheles stephensi* larval control using the fish in industrial tanks (9–180 m^2) was evaluated.

Results & conclusion: The fish was found in large numbers in estuarine waters, rivulets, backwaters and small dams in the districts of Kutch, Jamnagar, Patan and Anand in Gujarat. Mean daily consumption of larvae in laboratory was as follows: *An. stephensi* 128 ± 0.2 to 204 ± 6; *Cx. quinquefasciatus* 24 ± 4 to 58 ± 10; *Ae. aegypti* 43 ± 5 to 68 ± 2. In water tanks, *A. dispar* reduced 93% larval counts by Day 7 and 98% by Day 21 (p <0.01), showing high larval propensity. A consolidated information on bionomics, tolerance and physicochemical properties of water taken from natural habitats of *A. dispar* indicate its high larvivorous potential. Large-scale evaluation is warranted to evaluate its potential in vector-borne disease control.

Key words *Aphanius dispar;* geographical distribution; India; indigenous fish; killifish; larvivorous fishes; mosquito control

INTRODUCTION

Among the biological control agents of mosquitoes, fishes are the most extensively used species in several countries since the beginning of the twentieth century[1]. Certain exotic fishes such as *Poecilia reticulata, Gambusia affinis* and *Oreochromis mossambicus* have been used in various ecological conditions in India for mosquito control[2–4]. Use of these exotic fish has raised environmental concerns in view of their suspected adverse effects on local aquatic fauna[5]. Consequently, fish fauna surveys and evaluation of larvivorous potential of native fishes have been of high research priority in the area of biological control of vectors of disease. Probable larvivorous fishes of India were described in a historical review[6].

In India, a number of fish species have been surveyed in various states and their role in mosquito vector control has been evaluated[7–10]. However, the indigenous fish evaluated so far did not meet most of the biological characteristics of a potential larvivorous fish including their modest larval propensity and low breeding rate, which made them less amenable to their operational-scale use. Characteristics of a potential larvivorous fish are summarized elsewhere[11].

During an international scientific mission in 2001, one of the authors (RSY) reviewed the potential fish of the Eastern Mediterranean region including East Africa and discovered that *Aphanius dispar* (Rüppell) had a high potential of being a larvivorous fish, where a few small-scale field trials had shown its potential in mosquito control[12–13]. In the old Indian literature, there was a mention of the species as a killifish of western India[14]. In the present study, therefore, a further literature review and internet search were made to map and report geographical distribution of the species. We found that no work has been done to evaluate its potential in the control of Indian mosquito species under local conditions. An extensive fish fauna survey in Gujarat state was conducted to collect the fish for evaluation of larval propensity in the laboratory and on a small-scale in a field trial in Nadiad district, Gujarat. Based on these observations and analysis of physico-characteristics of water taken from natural habitats, information on the bionomics and tolerance limits of *A. dispar* was consolidated for future reference.

MATERIAL & METHODS

Fish collection

It was observed that the fish had a shoaling behaviour and large number of fishes tend to sustain in small and shallow water bodies. It prefer to dwell in water bodies with submerged aquatic vegetation. Batches of live fishes were collected in 2006 from shallow water pools often along rivulets in Rapar, District Kutch and in Gotarka, District Patan. Fishes were brought from natural habitats to the laboratory at Nadiad and acclimatized in aquarium containing tap water. Later in 2009 and 2010, fish surveys were also undertaken in the estuarine waters along the Gulf of Cambay and District Jamnagar in Gujarat. The fish were identified according to the description by Gopalakrishnan[11].

Bionomics and tolerance limits

Information on the biological characteristics of *A. dispar* was reviewed from the available literature on fishes and supplemented with field observations in this study. Water samples were collected from natural habitats of the fish in tight glass bottles. Three different water samples were analysed for salinity and other physicochemical properties at the Ipcowala Santram Institute of Biotechnology and Emerging Sciences, Dharmaj, District Anand, Gujarat.

Laboratory evaluation for larvivorous efficacy

In initial laboratory screening, samples of fish brought from natural habitats showed presence of larval heads in the gut content indicating that the fish had a natural propensity to prey upon mosquito larvae. To determine the larval propensity of *A. dispar,* laboratory evaluation was conducted on larvae of three vector mosquito species, viz. *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera : Culicidae). Adult fish of 2.2 to 3 cm size were kept in chlorine-free tap water in glass jars for acclimatization. Five fishes were released in a glass jar with 1 litre of water. A batch of 500 laboratory-reared larvae was added in the jar in the morning and larval consumption was observed every four hours. Total larval consumption was recorded at the end of 24 h when all remainder larvae removed. A fresh batch of 500 larvae was released in the jar and the tests were repeated for three consecutive days to establish the maximum devouring capacity of the fishes. Five replicates were run separately for each of I to IV instars of the three species. Mean daily consumption per fish was recorded for each larval instar and species separately.

Small-scale field evaluation

A small-scale field trial was conducted in 10 industrial tanks filled with water for use in cement article fabrication units in Village Kanjari, 10 km away from Nadiad. The size of the tanks ranged from 9 to 180 m^2 with an average water depth of 1 m and water pH of 7.5 to 9.0. Most of the tanks had algal blooms on water surface. *Anopheles stephensi* was the main species breeding with small presence of *Ae. aegypti* and *Cx. quinquefasciatus.* Pre-release larval density was recorded in all the 10 tanks. Tanks were allocated into two lots by matched pair randomization. In one lot of five tanks, fishes were released at 10 adult fish/m^2 area, while other five tanks were run as controls without fishes. A standard larval dipper of 300 ml was used for measuring larval densities. Larvae were collected from tanks with 5 dips and collected in enamel trays. Average larval density by mosquito species and tank was calculated on Day 0 (pre-application) and thereafter on Days 3, 7, 10, 14 and 21 after introduction of fish. Reduction in III and IV instars in experimental tanks was calculated using the formula below[15]:

$$\text{Percent reduction} = 100 - [(C_1 \times T_2)/C_2 \times T_1) \times 100]$$

Where, C_1 = Pre-release larval density in control tanks; C_2 = Post-release larval density in control tanks; T_1 = Pre-release larval density in fish tanks; and T_2 = Post-release larval density in fish tanks.

The statistical significance of differences in the mean daily consumption of the larvae of the three species by *A. dispar* were analysed using the Student's *t*-test. Data analysis was performed separately for I to IV instars of three species.

Geographical distribution

Apart from a literature review, an extensive internet search was made using the following key words, either alone or in combination: *Aphanius dispar*, biological control, fish, fish data base, killifish, larvivorous fish, malaria control, mosquito control, India, vector control, Eastern Mediterranean, East Africa.

RESULTS

The geographical distribution of *A. dispar* was reported in western India, Eastern Mediterranean (Pakistan, Iran, Iraq, United Arab Emirates, Saudi Arabia, Kuwait, Bahrain, Israel, Oman, and Yemen) and the East African countries (Djibouti, Eritrea, Egypt, Sudan, Ethiopia and Somalia) (Fig. 1). Knowledge so far on its distribution indicates that the fish is found usually in the coastal areas, while in Israel the species was introduced. In Gujarat, India, we captured the fish in large numbers in water bodies

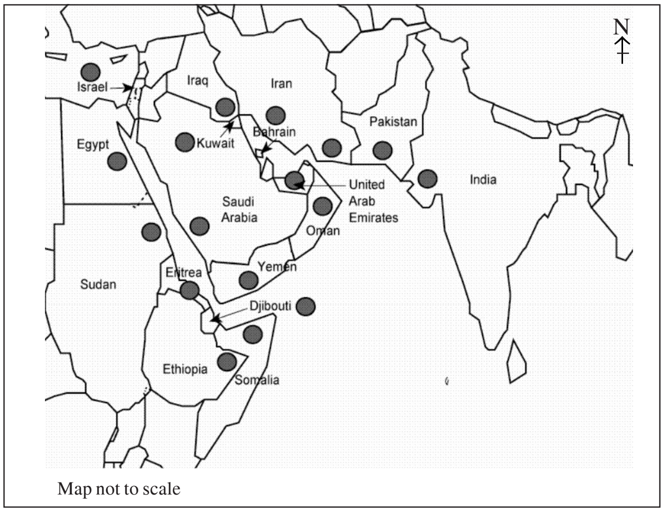

Fig. 1: Distribution of *Aphanius dispar* in the countries of the Eastern Mediterranean, East Africa and in India in the Gujarat state where the fish was found in its natural habitats in the present study.

such as estuarine waters, rivulets, backwaters and small dams in the Districts of Kutch, Jamnagar, Patan and Anand (estuarine areas in the Gulf of Cambay). A typical *A. dispar* male and female fish are shown in Fig. 2.

Larval feeding propensity of *A. dispar* showed that the fish consumed larvae of all the three mosquito species with varying preference (Table 1). Mean number of larvae consumed per fish per day was in the following order: *An. stephensi*, *Ae. aegypti,* and *Cx. quinquefasciatus*. Mean daily consumption of *An. stephensi* larvae ranged between 128±0.2 and 204±6; for *Ae. aegypti* it was 24±4 and 58 ± 10, and for *Cx. quinquefasciatus* it ranged between 43±5 and 68±2. Thus, among the three mosquito species, *A. dispar* showed a significantly greater feeding propensity for all instars of *An. stephensi* compared to either *Ae. aegypti* or *Cx. quinquefasciatus* larvae (p <0.001). The difference in the daily larval consumption rate for the two latter species was non-significant (p >0.2) for I instars but significantly different for II to IV instars (p <0.02). It was also noted that usually early instars were consumed in more number per day than the late instars of all the three species.

Figure 3 shows the larvivorous efficacy of *A. dispar* in industrial tanks with predominant breeding of *An. stephensi*. While the difference in the pre-release density of III and IV instars in fish tanks and control tanks was non-significant (p >0.2), there was a 93% reduction in larval density in fish tanks on Day 3 and 98% on Day 21 (p <0.01) relative to control.

Consolidated information on the bionomics and tolerance limits of the fish to various factors is given in Table 2, which is based on the observations in this study

Fig. 2: Adult *Aphanius dispar* male (top) and female (bottom) fish of 3.5 cm size. Males have dark crescent bands on the caudal fin, while females have vertical dark bands on the lateral side of the body.

Table 1. Mean daily consumption of mosquito larvae of main mosquito vector species by *Aphanius dispar* in a laboratory evaluation

Instars	Size of fishes (cm)	Mean no. of larvae consumed/ day ± SD		
		An. stephensi	*Ae. aegypti*	*Cx. quinquefasciatus*
I	2.4–2.8	188 ± 9[a]	67 ± 2[b]	58 ± 10[b]
II	2.5–3.0	204 ± 6[a]	68 ± 2[b]	53 ± 5[c]
III	2.2–2.7	193 ± 7[a]	52 ± 3[b]	27 ± 3[c]
IV	2.3–2.9	128 ± 0.2[a]	43 ± 5[b]	24 ± 4[c]

Figures with a different superscript in a given row were significantly different (p <0.02).

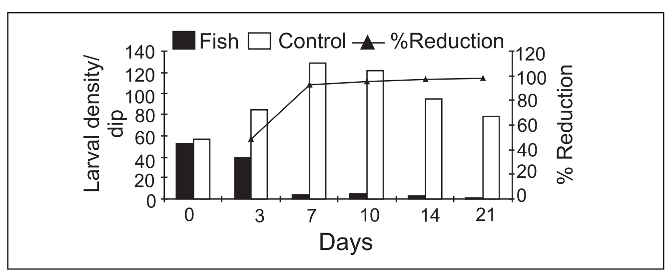

Fig. 3: Reduction in III and IV instars, predominantly of *An. stephensi*, by release of *Aphanius dispar* at 10 fishes/m^2 in industrial tanks. Pre-release larval densities (D-0) in fish tanks and control were not different significantly (p >0.2).

and available information through literature search. The physicochemical properties of water taken from natural habitats of fish showed a large range of salinity (12.3–108 ppt). Other parameters included water pH of 7.6–7.8, absence of free CO_2, and presence of bicarbonates (213–3035 ppm) and chlorides (4260–9585 ppm).

Table 2. Bionomics, behaviour, tolerance limits and geographical distribution of *Aphanius dispar* in India

Bionomics	
Family	Cyprinodontidae
Total length of adult fish	5–10 cm
Size of fry	Medium
Dimorphism of sexes	Intermediate; males with distinct dark crescent bands on caudal fins; females with black bands on the body
Type of spawning	Deposit eggs on plants and substratum
Incubation period	7–10 days
Resistance of eggs to desiccation	Not resistant
Rate of growth	Rapid
Food	Omnivorous including mosquito larvae; a voracious feeder
Position of mouth	Superior (a typical character of larvivorous fish)
Behaviour	Shoaling
Breeding potential	Year round and a prolific breeder
Temperature suitable for breeding	16–26°C
Water chemistry tolerance	
pH of water	6–9
Degree of hardness	Very hardy (tolerant to 12 g/L mineral concentration)
Salinity	Euryhaline; tolerant to brackish waters
Organic pollution	Resistant, hardy
Water temperature tolerance	Up to 40°C
Habitats	Associated with submerged vegetation or filamentous algae, very adaptable in freshwater and brackish waters, inhabits shallow coastal waters, lagoons, pools, ponds, estuaries, rivers, small-dams; lakes
Distribution in India	Gujarat state: Districts of Kutch, Jamnagar, Patan and Anand (Gulf of Cambay)

DISCUSSION

Although the focus of this study has been on distribution of *A. dispar* in various districts of Gujarat, for future reference we have consolidated the information on its geographical distribution in the coastal areas of India, Eastern Mediterranean and East Africa. In the laboratory evaluation, *A. dispar* showed high feeding propensity for larvae of Indian mosquito vectors, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The I and II instars were consumed more than the III and IV instars, probably due to their smaller size, although the fishes may have had consumed equal biomass. Among the three species used, *A. dispar* consumed more of *An. stephensi* larvae than other two species, as well as more of *Ae. aegypti* larvae than *Cx. quinquefasciatus*. Lower consumption of *Aedes* and *Culex* larvae may have been due to their larger size but also due to top-feeding *A. dispar*'s easy amenability of *Anopheles* larvae that also tend to occupy the top part of the water column.

In an earlier study, it was reported that under the laboratory conditions *A. dispar* was more successful than *G. affinis* in preying upon the III and IV instars and pupae, and that the two species could complement each other as mosquito control agents in different habitat conditions[16]. The results of the field trial showed that *A. dispar* is capable of controlling mosquito breeding in confined water bodies effectively within a fortnight of its application. An experimental study in Turkey showed high mosquito larval consumption by *A. chantrei* and recommended its use in biological control instead of *Gambusia* spp[17].

In this study, we found the fish present in waters with a wide range of salinity. Being a euryhaline fish (able to tolerate wide range of salinity), it can be used for mosquito control in brackish- and freshwater-habitats. Our experience of collecting the fishes from their natural habitats and storing them in freshwater tanks also builds confidence that the fish can be used in freshwaters beyond coastal areas for the purpose of vector control. Earlier studies have also shown that *A. dispar* is found in a wide range of salinities from fresh water to sea water and is capable of maintaining its body osmotic pressure and ionic concentration within a relatively narrow range against salinity changes from freshwater to sea water[18–21]. Its bionomics, tolerance to a range of salinity and pH, and capacity for prolific breeding in tropical waters make it a potential lavivorous fish in Indian conditions, especially in Gujarat state where it is a native fish and is found in abundance colonizing local coastal waters. Elsewhere, the species was also found to be tolerant to a wide range of temperature, salinity and photoperiod[22]. Another study reported a low growth of *A. dispar* at 40 ppt salinity, which increased towards the extreme ends of 8–56 ppt; there were significant increases in growth rates of adult *A.*

dispar with increase in feeding rates from 0 to 4% body weight/day or temperature from 18 to 23°C[23].

Malaria and dengue are major vector-borne diseases in Gujarat. A major development programme of the Government in the drought-affected areas has created thousands of check-dams, farm ponds and other rainwater harvesting systems, while water from Sardar Sarovar water resources development project is poised to irrigate a large area in the near future in semi-arid zone of Gujarat. Mosquito populations in arid and semi-arid areas are dependent on the availability of a limited number of aquatic habitats during the dry part of the year, thus, management of larval habitats and use of *A. dispar* in such confined waters can provide a local solution to abate the increasing risk of vector-borne diseases in such areas. Use of fish could reduce the reliance on insecticides and may provide a cost-effective, environmentally safe and target-specific vector control tool.

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REFERENCES

1. Gerberich JB, Laird M. A bibliography of papers relating to the control of mosquitoes by the use of fish: an annotated bibliography for the years 1901–1966. *FAO Fisheries Tech Paper* 1968; *75:* p. 70.
2. Sitaraman NL, Mahadevan S, Asamidas S. Biological control of *An. stephensi* larvae in wells by *Poecilia reticulata* in Greater Hyderabad city. *J Commun Dis* 1976; *8:* 315–9.
3. Chand SK, Yadav RS. Use of *Oreochromis mossambicus* (Peters) in controlling mosquito breeding in cow dung pits. In: Sharma VP, Ghosh A, editors. *Larvivorous fishes of inland ecosystems.* Proceedings of the MRC-CICFRI workshop, New Delhi 27–28 September 1989. Delhi: Malaria Research Centre (ICMR) 1994; p. 115–20.
4. Prasad RN, Das MK, Virk KJ, Haq S. Use of *Gambusia affinis* on large scale for control of malaria vector: An overview. In: Sharma VP, Ghosh A, editors. *Larvivorous fishes of inland ecosystems.* Proceedings of the MRC-CICFRI workshop, New Delhi 27–28 September 1989. Delhi: Malaria Research Centre (ICMR) 1994; p. 69–81.
5. Hulbert SH, Zedier J, Fairbanks D. Ecosystem alteration by mosquito fish (*Gambusia affinis*) predation. *Science* 1972; *175:* 639–41.
6. Prasad B, Hora SL. A general review of the probable larvivorous fishes of India. *Records Mal Survey Ind* 1936; *6*(4): 631–48.
7. Haq S, Yadav RS. Fish fauna of district Raigad, Maharashtra with particular reference to mosquito larvivorous species. *Indian J Malariol* 1997; *34:* 213–16.
8. Haq S, Prasad H, Prasad RN, Sharma T. Availability and utility of local fishes of Shahjahanpur for mosquito control. *Indian J Malariol* 1993; *30:* 1–8.
9. Yadav RS, Padhan K, Sharma VP. Fishes of district Sundargarh, Orissa, with special reference to their potential in mosquito control. *Indian J Malariol* 1992; *29:* 225–33.
10. Yadav RS, Das MK. Role of *Danio* and *Oryzias* fishes in the control of mosquito breeding in rice fields. In: Sharma VP, Ghosh A, editors. *Larvivorous fishes of inland ecosystems.* Proceedings of the MRC-CICFRI workshop, New Delhi 27–28 September 1989. Delhi: Malaria Research Centre (ICMR); 1994; p. 61–8.
11. Gopalakrishnan V. Table for the identification of Indian freshwater fishes, with description of certain families and observations on the relative utility of the probable larvivorous fishes of India. Health Bulletin No. 12 (Revised edition). Delhi: Manager of Publications, Government of India 1972; p. 1–56.
12. Ataur-Rahim M. Observations on *Aphanius dispar* (Rüppell, 1828), a mosquito larvivorous fish in Riyadh, Saudi Arabia. *Ann Trop Med Parasitol* 1981; *75:* 359–62.
13. Louis JP, Albert JP. Malaria in the Republic of Djibouti. Strategy for control using a biological antilarval campaign: indigenous larvivorous fish (*Aphanius dispar*) and bacterial toxins. *Med Trop* 1988; *48:* 127–31.
14. Fletcher M, Teklehaimanot A, Yemane G. Control of mosquito larvae in the port city of Assab by an indigenous larvivorous fish, *Aphanius dispar*. *Acta Trop* 1992; *52:* 155–66.
15. Mulla MS, Nodlard RL, Eanara DM, Darwazeh HA, Mckean DW. Control of chironomid midges in recreational lakes. *J Econ Entomol* 1971; *264:* 300–7.
16. Homski D, Goren M, Gasith A. Comparative evaluation of the larvivorous fish *Gambusia affinis* and *Aphanius dispar* as mosquito control agents. *Hydrobiol* 1994; *284:* 137–46.
17. Yildirim O, Karacuha A. A preliminary study on determination of *Aphanius chantrei's* feeding behaviour on mosquito larvae. *Acta Trop* 2007; *102:* 172–5.
18. Lotan R. Sodium, chloride and water balance in the euryhaline teleost *Aphanius dispar* (Rüppell) (Cyprinodontidae). *Zvergl Physiologie* 1969; *65:* 455–62.
19. Lotan R. Osmotic adjustment in the euryhaline teleost *Aphanius dispar* (Cyprinodontidae). *Z vergl Physiologie.* 1971; *75:* 383–7.
20. Lotan R, Skadhauge E. Intestinal salt and water transport in a euryhaline teleost, *Aphanius dispar* (Cyprinodontidae). *Comp Biochem Physiol* 1972; *42A:* 303–10.
21. Skadhauge E, Lotan R. Drinking rate and oxygen consumption in the euryhaline teleost *Aphanius dispar* in waters of high salinity. *J Exp Biol* 1974; *60:* 547–56.
22. Frenkel V, Goren M. Some environmental factors affecting the reproduction of *Aphanius dispar* (Rüppell 1828). *Hydrobiol* 1997; *347:* 197–207, DOI: 10.1023/A:1003000225293.
23. Frenkel V, Goren M. Factors affecting growth of killifish, *Aphanius dispar*, a potential biological control of mosquitoes. *Aquaculture* 2000; *184:* 255–65.

Correspondence to: Dr Sarfarazul Haq, National Institute of Malaria Research Field Unit, Civil Hospital, Nadiad–387 001, Gujarat, India.
E-mail: sarfaraz_55@rediffmail.com

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