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

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**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 m²) 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 century¹. Certain exotic fishes such as *Poecilia reticulata*, *Gambusia affinis* and *Oreochromis mossambicus* have been used in various ecological conditions in India for mosquito control²–⁴. Use of these exotic fish has raised environmental concerns in view of their suspected adverse effects on local aquatic fauna⁵. 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 review⁶.

In India, a number of fish species have been surveyed in various states and their role in mosquito vector control has been evaluated⁷–¹⁰. 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 elsewhere¹¹.

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 control¹²–¹³. In the old Indian literature, there was a mention of the species as a killifish of western India¹⁴. 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 Gopalakrishnan11.

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 m² 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/m² 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 below15:

\[
\text{Percent reduction} = 100 - \left[\frac{(C_1 \times T_2)}{(C_2 \times T_1)}\right] \times 100
\]

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
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.

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

<table>
<thead>
<tr>
<th>Instars</th>
<th>Size of fishes (cm)</th>
<th>Mean no. of larvae consumed/ day ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>An. stephensi</td>
<td>Ae. aegypti</td>
</tr>
<tr>
<td>I</td>
<td>2.4–2.8</td>
<td>188 ± 9a</td>
</tr>
<tr>
<td>II</td>
<td>2.5–3.0</td>
<td>204 ± 6a</td>
</tr>
<tr>
<td>III</td>
<td>2.2–2.7</td>
<td>193 ± 7a</td>
</tr>
<tr>
<td>IV</td>
<td>2.3–2.9</td>
<td>128 ± 0.2a</td>
</tr>
</tbody>
</table>

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

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 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 CO₂, and presence of bicarbonates (213–3035 ppm) and chlorides (4260–9585 ppm).
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. 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.

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. 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. 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.

<table>
<thead>
<tr>
<th>Table 2. Bionomics, behaviour, tolerance limits and geographical distribution of <em>Aphanius dispar</em> in India</th>
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</thead>
<tbody>
<tr>
<td><strong>Bionomics</strong></td>
</tr>
<tr>
<td>Family</td>
</tr>
<tr>
<td>Total length of adult fish</td>
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<tr>
<td>Size of fry</td>
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<tr>
<td>Dimorphism of sexes</td>
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<tr>
<td>Type of spawning</td>
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<td>Incubation period</td>
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<td>Resistance of eggs to desiccation</td>
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<td>Rate of growth</td>
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<tr>
<td>Food</td>
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<td>Position of mouth</td>
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<tr>
<td>Behaviour</td>
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<td>Breeding potential</td>
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<td>Temperature suitable for breeding</td>
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<tr>
<td><strong>Water chemistry tolerance</strong></td>
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<tr>
<td>pH of water</td>
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<tr>
<td>Degree of hardness</td>
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<tr>
<td>Salinity</td>
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<tr>
<td>Organic pollution</td>
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<tr>
<td>Water temperature tolerance</td>
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<tr>
<td>Habitats</td>
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<tr>
<td>Distribution in India</td>
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**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**


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