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Role of ornamental fishes to control *Aedes albopictus* larvae population

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Abstract
Aedes albopictus is a potential vector that is responsible for severe outbreaks of dengue and DHF. Implementing traditional methods such as insecticides and mosquito repellents are harmful to the environment and to human health. In the present study, the rate at which *Aedes albopictus* larvae were consumed as well as the other feeding preferences of five ornamental fishes (Guppy, Molly fish, Betta fish, Gold fish, and Angel fish) were noted and compared. While Molly fish, Fighter, and Guppy considerably prefer *Aedes albopictus* larvae over the other food item, Gold fish and Angel fish exhibit good efficacy toward both types of food. In the last decade of little works (use of ornamental fish) have been done to control *Aedes* mosquito population. Therefore, a preliminary attempt has been undertaken to include ornamental fishes as vector controlling agent.

Keywords: *Aedes albopictus*, ornamental fish, biological control, feeding preference, dengue hemorrhagic fever

1. Introduction
Spreading of vector borne diseases has become a significant threat to world. Vector-borne diseases including dengue, chikungunya, and Zika viruses are transmitted by *Aedes* mosquito (*Aedes albopictus*). In the past decades the incidence of dengue has grown exponentially around the world. Recent resurgence of dengue and Dengue Hemorrhagic Fever (DHF) is a raging problem in Urban and Suburban areas of West Bengal. It has been reported [1] that the West Bengal faces a new threat in the form of dengue and DHF as the cases (nearly 70%) are increasing day by day in Kolkata and neighboring districts (North 24 Parganas, Murshidabad and Malda). Many scientists [2, 3] reported that using of larvicides, insecticides; mosquito repellents can not only create health problems for human beings but also has an adverse effect on non-target populations. As chemical control is costly and time consuming, therefore biological control is effective for reduction of *Aedes* mosquito population. Various lines of data [4, 5, 6] indicated that biological mosquito control programs have been lucrative in controlling *Aedes aegypti* populations. Since the early 1900s, larvivorous fishes are being widely used as biological mosquito control agents in all over the world [7]. Use of several larvivorous ornamental fishes is an effective, sustainable and ecofriendly mosquito control strategy as compared to use chemical (insecticides). Several lines of data [8, 9, 10, 11, 12, 13, 14] reported that use of some indigenous larvivorous fishes have been very successful for controlling mosquito species population. Numerous data [15, 16, 17, 18] suggested that biological control using larvivorous fishes have been also effectively used in malaria control programmes in the 20th century. Large numbers of larvivorous fishes (surface feeders and carnivorous in habit) show preference for mosquito larvae even in the presence of other food materials [16]. Local fish (*Gambusia* sp., *Aphanius* sp. and *Tilapia* sp.) have been properly utilized to control mosquito larvae [19]. Earlier it has been reported [20] that keeping an ornamental fish, not only provides aesthetic beauty but also provide financial benefits. Ornamental fishes like *Poecilia reticulata* (Guppy), *Poecilia sphenops* (Black Molly), *Betta splendens* (Betta fish), *Carassius auratus* (Gold Fish), *Pterophyllum scalare* (Angel Fish) are known predators of *Aedes albopictus* larvae.

Betta and Angel fish are carnivorous they feed on *Tubifex* worms and mosquito larvae. Guppy and Swordtails are omnivorous; feed on foods like mosquito larvae, bloodworms, and daphnia. Furthermore, the black molly consumes mainly with fine plant-based flake foods, algae and larvae. Gold fish is a good consumer of mosquito larvae. Their preference for live food is noticeably higher than their preference for artificial food [21]. Therefore, to observe an effect of ornamental fish to control mosquito larvae *(Aedes albopictus*), we have collected *Aedes albopictus* larvae from different areas (Hooghly, Howrah and South 24 Parganas) of West Bengal, where the population of *Aedes albopictus* larvae is significantly higher. In West Bengal, a very little work has been done to control *Aedes* mosquito with the aid of ornamental fishes, therefore a preliminary attempt has been conducted to observe the role of ornamental fishes as larvicidal agents to control *Aedes albopictus* population in the presence of alternative food.

2. Materials and Methods

2.1 Sites of collection

Fig 1: Map showing the collecting sites

2.2 Information on the collection sites

Table 1: Information on the collection sites

District	Site of Collections	Latitude	Longitude
Hooghly	Sheoraphuli	22.7705°N	88.3220°E
Howrah	Bally	22.6497°N	88.3386°E
South 24 Parganas	Canning	22.3104°N	88.6579°E

2.3 Collection of larvae, fish pellets and Fishes

Different instars of *Aedes albopictus* larvae were collected from Howrah, Hooghly and South 24 Parganas in West Bengal. Primary sources of collection include plastic containers, metal drums, PVC water reservoirs and any stagnant water source.

Fig. 2: *Aedes albopictus* larvae

Fig 3: Various Collection Sites of *Aedes albopictus* larvae

2.4 Collection of Fish pellets and Fishes

Carassius auratus (Goldfish), *Pterophyllum scalare* (Angelfish), *Betta splendens* (Betta fish), *Poecilia sphenops* (Black molly) and *Poecilia reticulata* (Guppy), all of nearly same age group and the fish pellets were purchased from ornamental fish shops. For one week, the fish were kept in 1L beakers under laboratory condition.

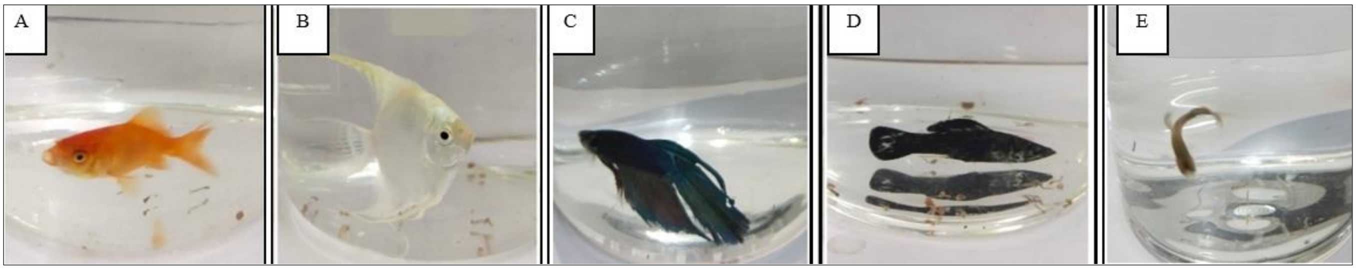

Fig 4: Five ornamental fish species [A] Goldfish [B] Angelfish [C] Betta fish [D] Black molly [E] Guppy

2.5 Experimental design

For each of the fish species, two distinct sets of trials using two different combinations of food were conducted at room temperature. In order to reliably measure the larvicidal activity of the fishes toward Aedes albopictus larvae, each experiment was conducted with seven repetitions to ensure that the fishes had enough time to become accustomed to the meal combinations provided before beginning a new set of combinations. In the first set of experiment, forty (40) *Aedes albopictus* larvae of all instar stages were given to each fish and were observed for 3 hours. In the second set, each fish was given 20 larvae with 20 artificial fish pellets and the fish were then left to be watched for three hours. The amount of ingested larvae and fish pellets were recorded.

2.6 Statistical Analysis

For each set of experiment, two-way ANOVA was run for five fish species and combination of food using Graphpad Prism (Version-8). Here, statistical differences were set at $p<0.05$.

3. Results and Discussion

3.1 Results

The first experiment was carried out to determine the food preference of five fishes towards *Aedes albopictus* larvae. The result (Table-2, Fig.5) indicated that Angle fish, Gold fish and Betta fish showed greater larvicidal efficacy than Molly and Guppy ($p<0.0001$) in absence of other food material. Being smaller in size, Guppy preferred I and II instar larvae whereas rest of the fishes favoured all instar larvae.

Table 2: The mean consumption rate of five distinct fishes against *Aedes albopictus* larvae at the 5% level

Name of Fish	Consumption Rate of *Aedes albopictus* Larvae (Mean ± SD)
Pterophyllum scalare (Angel Fish)	39.71±1.857
Carassius auratus (Gold Fish)	38.28±1.108
Betta splendens (Betta fish)	36.57±1.409
Poecilia sphenops (Molly fish)	29.43±2.089
Poecilia reticulata (Guppy)	24.43±1.473

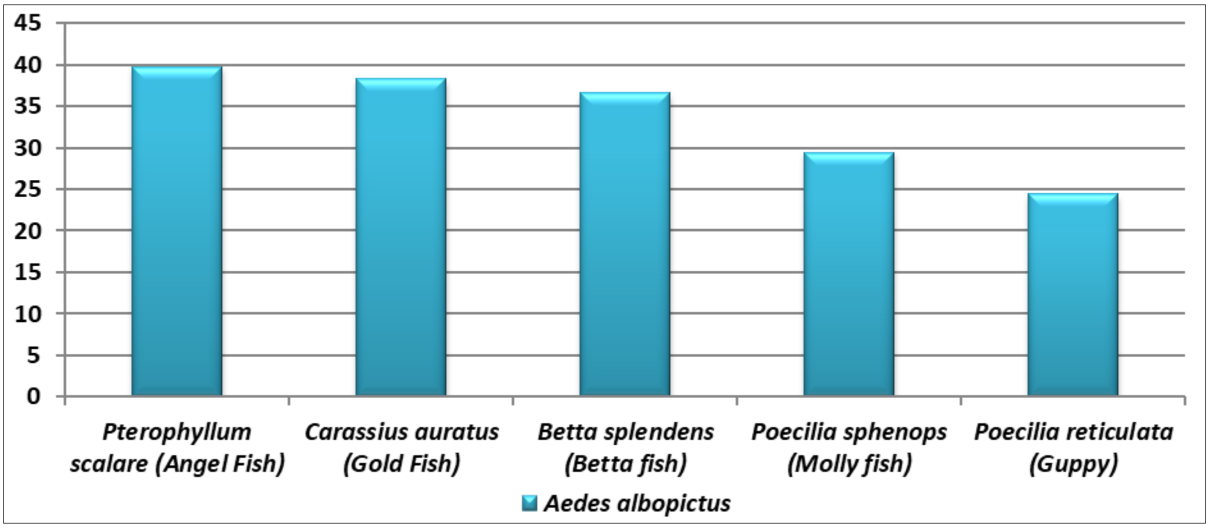

Fig 5: The mean consumption rate of five distinct fishes *against Aedes albopictus* larvae

The second experiment was carried out to learn about the food selection behavior of six fishes between Aedes albopictus larvae and fish pellets. It has been observed (Table-3 and Fig.6) that Angel fish and Gold fish consumed both the food items almost equally within the stipulated time. Whereas, Betta, Molly and Guppy preferred *Aedes albopictus* larvae more than the fish pellets. The Two way ANOVA result showed that the interaction between *Aedes albopictus* larvae and fish pellets are extremely significant at 0.05 level confidence (Table-4).

Table 3: The mean consumption rate of five distinct fishes against *Aedes albopictus* larvae with respect tofish pellets at the 5% level.

Name of Fish	Consumption Rate (Mean ± SD)	
	***Aedes albopictus* Larvae**	**Fish pellets**
Betta splendens (Betta fish)	17.57±1.04	1.83±0.55
Poecilia reticulata (Guppy)	12.28±1.23	1.43±0.78
Poecilia sphenops (Molly fish)	14.57±0.87	10.86±0.92
Carassius auratus (Gold Fish)	19.43±0.54	17.57±0.67
Pterophyllum scalare (Angel Fish)	20±0	19.29±0.52

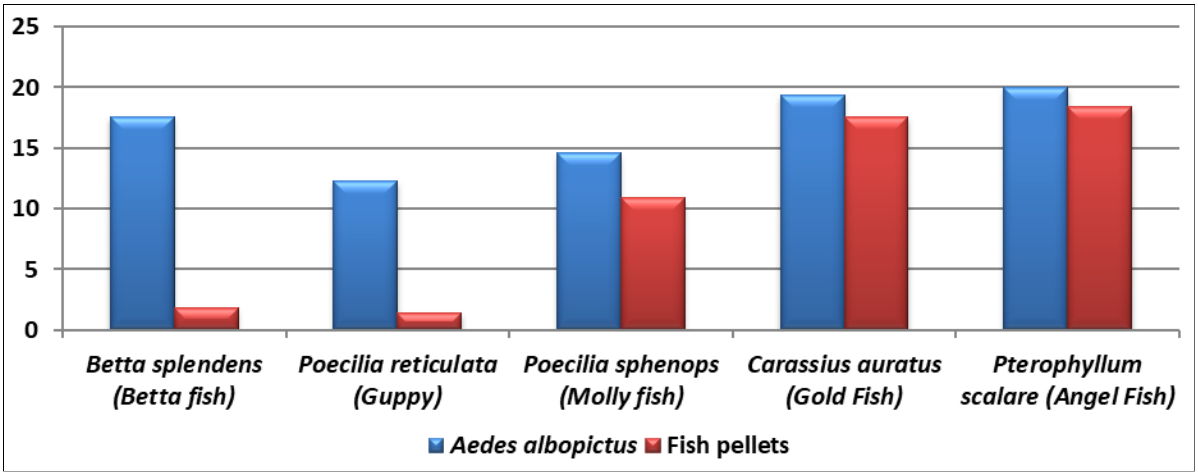

Fig 6: Food preference of five distinct types of fishes for two distinct types of food items when provided heterogeneously.

Table 4: Two way ANOVA of the five distinct fishes on consumption of *Aedes albopictus* larvae and fish pellets

	Sum of Squares	df	Mean Square	F value	P value	Significant
Fishes	1705	4	426.3	998.4	*p*<0.0001	Yes
Aedes albopictus larvae and fish pellets	755.7	1	755.7	204.8	*p*<0.0001	Yes
Interaction	583.3	4	186.9	145.8	*p*<0.0001	Yes

*Significant at 0.05 level confidence

3.2 Discussion

Our present observations indicated that all five fishes show significant higher preference for *Aedes albopictus* larvae than other food item (Table-2 and Fig.5). Moreover, when *Aedes albopictus* larvae and fish pellets were supplied together, Angel fish (*Pterophyllum scalare)* and Gold fish (*Carassius auratus*) consumed almost equally both the food items within the given time limit, Molly fish (*Poecilia sphenops)* moderately consumed both type of food, but, Betta (*Betta splendens)* and Guppy (*Poecilia reticulata*) preferred *Aedes albopictus* larvae more than the fish pellets (Table-3, Fig.6 and Table-4). Meraldo and Pecora (2017) [22] reported that Angel fish can be used as a potential biocontrolling agent against *Aedes* larvae. Earlier studies [26] suggested that ornamental fishes specially Gold fish exhibit excellent larvivorous potentials to control mosquito population. Recently in our laboratory similar findings [23, 24] were well documented. Furthermore, Mannaa *et al*., (2008) [27] in his observation suggested that *Poecilia reticulata* can consume 65-84 IV instar *Culex* larvae in a period of 3 hours. It has also been reported (Sumithra *et al*., 2014 [3] and Ghosh *et al*., 2011 [25]) that *Poecilia sphenops* can be used in mosquito control programme. Therefore, ornamental fish have high implications in mosquito larvae control system and the strategy is also very simple and cost effective.

4. Conclusion

The presence of ornamental fish in mosquito breeding habitat is not only helps to control vector population but also maintain ecosystem. All the five fishes i.e.; *Betta splendens* (Betta fish), *Poecilia reticulata* (Guppy), *Poecilia sphenops* (Black Molly), *Carassius auratus* (Gold Fish), *Pterophyllum scalare* (Angel Fish) have shown higher mosquito larvicidal efficacy in the presence of other artificial food. As larvivorous fish can control *Aedes albopictus* larvae population and thereby can easily prevent the transmission of vector borne diseases. As mosquitoes are becoming resistant towards the chemical insecticides, larvivorous fishes can be used to reduce the number of *Aedes albopictus* larvae population. Therefore, it is concluded that ornamental fishes can be used in vector control programme as an alternative way when other potential modes fail.

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