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A comparative study of consumption of *Aedes aegypti* and *Aedes albopictus* larvae by ornamental fishes to control their population in West Bengal

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Abstract
Aedes aegypti and *Aedes albopictus* are the carriers of a variety of diseases such as dengue, dengue hemorrhagic fever (DHF), chikungunya, zika virus, and yellow fever. An affordable way of vector management that effectively suppresses the mosquito population for a long time is the release of larvivorous fish. In this work, ornamental fish species are identified, and their potential larvicidal efficacy for biological control of *Aedes* mosquito larvae is assessed. The consumption rates of four ornamental fish species (Gold fish, Betta fish, Molly fish, and Guppy) for *Aedes aegypti* and *Aedes albopictus* larvae have been observed in the present study, and a comparison between the mean consumption rates of the four fish species for *Ae. aegypti* and *Ae. albopictus* has been recorded. The observation indicated practically no variance, and the intake rates for both types of larvae were almost same for the four species of fish. In contrast to guppy fish, the findings indicated that Goldfish, Black molly, and Betta fish had very high feeding rates. Therefore, in the near future, these fish can be thought of as efficient biological control agents for both *Aedes aegypti* and *Aedes albopictus* larvae.

Keywords: *Aedes aegypti*, *Aedes albopictus*, ornamental fish, biological control, food preference, larvicidal efficacy

Introduction

Mosquitoes are essential insects not just as nuisance biters, but also as disease vectors. Several mosquito species specially *Aedes aegypti* and *Aedes albopictus*, are responsible for the transmission of several important viral infections, including dengue, dengue hemorrhagic fever (DHF), yellow fever, and chikungunya. These diseases have become major public health problems in the world's tropical and subtropical climates [1]. Dengue fever is one of the world's fastest spreading diseases, endemic in more than 120 countries, and up to 390 million cases are anticipated to occur each year [2]. Dengue-related deaths are significantly less common, although the projected number of apparent cases worldwide in 2013 was 58.4 million [3]. Yellow fever, on the other hand, is currently endemic in 47 countries and fatalities between 29,000 and 60,000 people each year [4]. Therefore the only ways to prevent dengue transmission and reducing the disease's burden should be the control of mosquitoes, particularly *Aedes aegypti* and *Aedes albopictus*, or eliminating human-vector interaction.

The African subcontinent is most likely the origin of *Aedes aegypti*. It later spread to tropical, subtropical, and temperate areas. Currently, this species can be found in Africa, South and Central America, portions of North America, the Middle East, Southeast Asia, and Oceania, including Northern Australia [5]. It is primarily an urban mosquito that bites during the day and lays drought-resistant eggs [6]. They also have a tendency to breed in containers and tiny areas, such as between enormous leaves and stems of vegetation. Female *Aedes aegypti* mosquitos prefer to feed on humans who are in enclosed places, such as houses [7].

Aedes albopictus, like *Aedes aegypti*, has been increasingly intertwined with urban and peri-urban areas as its geographic range has grown [8]. The species has become increasingly capable of exploiting human-made container habitat and human blood meal hosts inside these landscapes [6].

It can resist a wider range of temperatures and prefers cool climates. As a result, this mosquito species may now survive in more temperate climates [7]. *Aedes albopictus* mosquitos are currently found throughout Africa, Asia, South America, and the Pacific and Indian Ocean islands [9]. *Ae. albopictus* feeds during the day, preferring vegetation-rich habitats that have a more urban and semi-urban appearance [10]. Numerous laboratory studies show that *Ae. albopictus* can be equally able to acquire and transmit pathogens as *Ae. aegypti* for a variety of arboviruses, including chikungunya and Zika [11, 12, 13].

For reducing the risk of or preventing epidemics, or for responding to outbreaks, respectively, dengue vector control may be aimed against the aquatic larval stages or the adult female mosquito [14]. Adult mosquitoes are typically targeted by insecticide fogging or space spraying in response to confirmed dengue transmission, with the aim of reducing biting and transmission as quickly as possible [15]. In addition, there is considerable evidence that insecticide-resistant mosquitoes are emerging in dengue-endemic areas [16, 17].

In the 20th century, biological control, notably the utilization of larvivorous fish, was vital to malaria control programmes, especially in urban and periurban regions for immediate application in industrialised and developing countries. It has a very significant role to fulfil in the integrated control methods when both fish or other biotic agents and pesticides have distinct roles to play [18]. The WHO has continued to include the use of larvivorous fish as a potential method of reducing dengue vectors; however the capability of fish in preventing or controlling dengue is likely to differ from that of malaria. This is primarily due to the fact that *Aedes* spp. vectors of dengue typically breed in discrete man-made containers and household water tanks, in contrast to the majority of malaria vectors [2]. Job (1940) [19] states that larvivorous fish need to be small, hardy, and agile in shallow water among dense weeds, which are find to be ideal breeding sites of mosquitoes. They must be able to survive in both deep and shallow waters, as well as in drinking water tanks and pools, without contaminating the water, and they must be drought-resistant and must be able to survive harsh handling and long range transportation. Fish that survive must be prolific breeders with a short life cycle. In confined waters, they must effectively and freely reproduce. Even in the presence of other food sources, larvivorous fish should prefer mosquito larvae and be surface feeders and carnivorous by nature. Over many years, various fish species have been studied for their potential to reduce mosquito populations. These include fish reared for human food, such as species of tilapia (family Cichlidae), as well as larvivorous members of the Poeciliidae family (often known as "guppies") [20, 21, 22, 23]. Numerous researchers have investigated the larvivorous capabilities of local fishes [24, 25]. The ornamental tanks, on the other hand, make for the perfect mosquito breeding environment. These ornamental fish not only have a huge amount of potential for introduction in ornamental tanks, where they would not only provide aesthetic attractiveness but also control mosquito breeding [26]. According to Chandra *et al*. (2008) [18], Câmara *et al*. (2017) [27], Sumithra *et al*. (2014) [28], some ornamental fish species, such as *Poecilia reticulata* (Guppy), *Poecilia sphenops* (Black Molly), *Carassius auratus* (Gold Fish), and *Betta splendens* (Betta Fish), are used as larvivorous However, there hasn't been much research done yet to evaluate the potential of ornamental fish for mosquito larval control. Therefore, the objective of the present laboratory study was to evaluate how effectively these ornamental fishes controlled the populations of *Aedes aegypti* and *Aedes albopictus* mosquitoes by eliminating their larvae respectively in the presence of alternative food, and to determine whether there was a difference in the larval consumption rates of these ornamental fishes among the two mosquito species.

Materials and Methods

Collection Sites

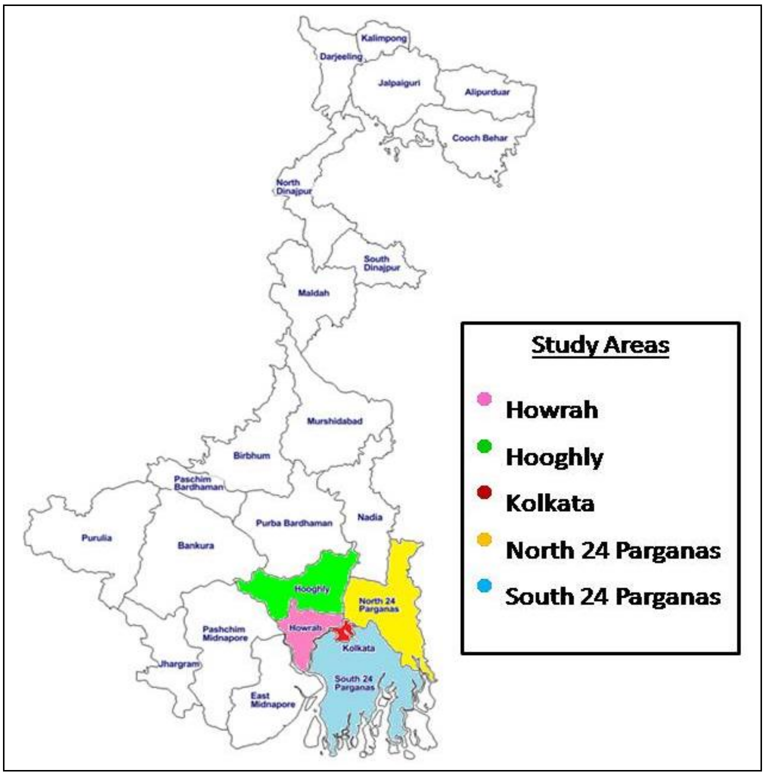

Fig 1: Map representing the collection sites of study area

Description of collection sites

Table 1: Description of collection sites

District	Site of Collections	Collected Species	Latitude	Longitude
Howrah	Shibpur	*Aedes albopictus*	22.5713°N	88.3109°E
	Salkia	*Aedes aegypti*	22.6013°N	88.3313°E
Hooghly	Serampore	*Aedes albopictus*	22.7505°N	88.3406°E
	Uttarpara	*Aedes aegypti*	22.6701°N	88.3355°E
Kolkata	New Alipore	*Aedes aegypti*	22.5090°N	88.3329°E
	Hazra	*Aedes aegypti*	22.5228°N	88.3500°E
North 24 Parganas	Dumdum	*Aedes aegypti*	22.6420°N	88.4312°E
	Barasat	*Aedes aegypti*	22.7248°N	88.4789°E
South 24 Parganas	Diamond Harbour	*Aedes albopictus*	22.1927°N	88.1895°E

Collection of Larvae

Larvae of different instar stages of *Aedes aegypti* and *Aedes albopictus* were collected from suspected breeding sites of Howrah, Hooghly, Kolkata, North 24 Parganas, and South 24 Parganas in West Bengal. Plastic containers, discarded jars, tyres, discarded bottles, PVC water reservoirs were considered as primary and major sources of collection.

Fig 2: Various collection sites of *Aedes* larvae

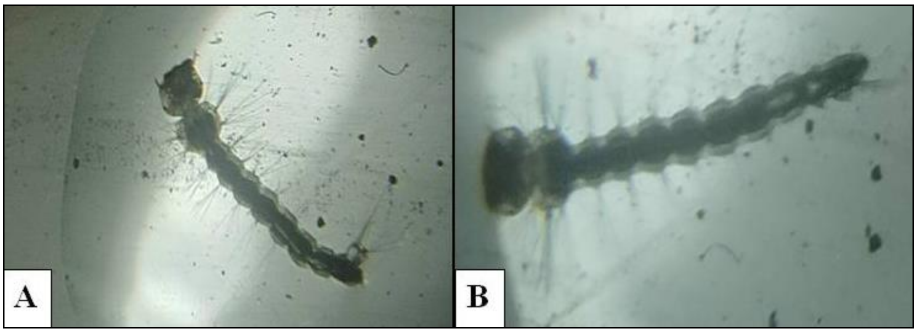

Fig 3: [A] *Aedes aegypti* larva [B] *Aedes albopictus* larva

Collection of Fish, Fish pellets and *Tubifex* sp.

Four species of ornamental fishes, namely *Carassius auratus* (Goldfish) of 5.8 cm length, *Betta splendens* (Betta fish) of 6.9 cm length, *Poecilia reticulata* (Guppy) of 2.7 cm length, and *Poecilia sphenops* (Black molly) of 3.2 cm length, all under same age group were purchased from ornamental fish shops of Kolkata and Howrah area. Artificial fish pellets (Optimum) and live *Tubifex* sp. were collected from local aquarium shops in Serampore. Fishes were kept in four 5L glass tanks under the laboratory condition for seven days were supplied with commercial feed. *Tubifex* sp. were kept in plastic tray submerged in water, and water was changed on a daily basis for both the fishes and *Tubifex* sp.

Fig 4: [A] *Carassius auratus* (Gold Fish), [B] *Betta splendens* (Betta fish), [C] *Poecilia reticulata* (Guppy) [D] *Poecilia sphenops* (Black Molly)

Experimental Design

The fishes were kept for 24 hours without any type of food supply. This was done to make sure that they were in fasting condition before starting the experiments. For each fish, three different sets of experiments were done. In the first set, each fish was given 50 *Aedes aegypti* larvae, 50 *Tubifex* sp., and 50 optimum fish pellets; on the second set, each fish was given 50 *Aedes albopictus* larvae, 50 *Tubifex* sp. and 50 optimum fish pellets and on the third set of experiment, 50 *Aedes aegypti* and 50 *Aedes albopictus* larvae were introduced to each fish species and were observed for 4 hours. The temperature was set at 25 °C.

Each experiment was repeated for ten days to make sure that there is a sufficient time period for the fishes to get used to of the food choice given before starting a new set of combination, so that the larvicidal activity towards the *Aedes aegypti* and *Aedes albopictus* larvae could be observed properly. The mean consumption rate of four fishes for *Ae. aegypti* and *Ae. albopictus* were taken and observed if any variation exists in the feeding rate of the four fishes for the two species of *Aedes*.

Statistical Analysis

Two-way ANOVA was performed for each set of experiments followed by Tukey's multiple comparison test for four fish species for ten days of observation using GraphPad Prism (Version-8). Statistical differences were set at $p<0.05$.

Result and Discussion

Result

The results obtained from the first set of experiment showed significantly higher preference ($p<0.0001$) for *Ae. aegypti* larvae than the other two food options for all the fish species. All of the fish species preferred live food than artificial food pellets, but the preference for *Aedes aegypti* larvae was higher than *Tubifex* sp. Among the four fishes, Goldfish, Black Molly and Betta fish devoured almost all the larvae in every replicate of the experiments, whereas the feeding rate of guppy was significantly low as compared to the other counterparts. The two way ANOVA showed the results are highly significant at 5% level of confidence.

Table 2: The mean consumption rate of *Aedes aegypti* larvae, *Tubifex* sp. and Optimum fish pellets by four different fishes at the 5% level.

Name of Fish	Consumption Rate (Mean ± SD)		
	Aedes aegypti Larvae	***Tubifex* sp.**	**Optimum Fish pellets**
Carassius auratus (Gold Fish)	48.6± 2.95	45.2 ± 5.21	18.9 ± 4.57
Betta splendens (Betta fish)	45.7 ± 2.62	39.2 ± 3.55	1.3 ± 3.07
Poecilia sphenops (Molly fish)	43.2 ± 4.69	39.9 ± 4.57	11 ± 3.58
Poecilia reticulata (Guppy)	38.4 ± 3.72	21.8 ± 4.21	1.1 ± 2.15

Table 3: Two way ANOVA of the different fishes on consumption of *Aedes aegypti* larvae, *Tubifex sp.* and Optimum fish pellets

	Sum of Squares	df	Mean Square	F value	P value	Significant
Fish	4540	3	1513	407.9	P<0.0001	Yes
Aedes aegypti larvae , *Tubifex* sp. and Optimum fish pellets	28716	2	14358	3659	P<0.0001	Yes
Interaction	1329	6	221.5	56.46	P<0.0001	Yes

*Significant at 0.05 level confidence

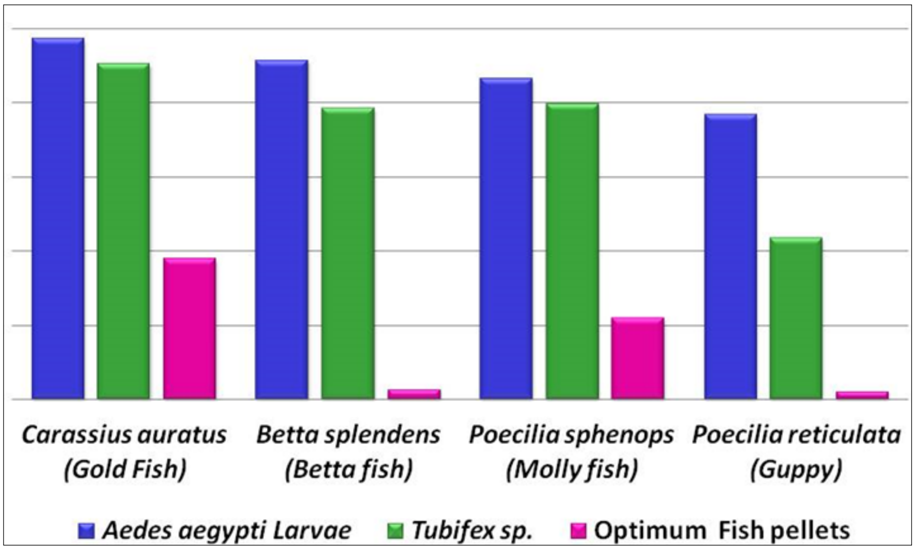

Fig 5: Food preference of four different types of fish for three different types of food items when supplied heterogeneously.

In the second set of experiment also, when the larvae choice was changed, the results showed significantly higher preference ($p<0.0001$) for *Ae. albopictus* larvae than the other two food options for all the fish species. Here also we observed greater preference for *Ae. albopictus* larvae than the live *Tubifex* sp. species for all the four fishes. Among the four fishes, Goldfish, Black Molly and Betta fish devoured almost all the larvae in the experiments, but here also, the feeding rate of guppy was comparatively low. The two way ANOVA showed the results are significant at 5% level of confidence.

Table 4: The mean consumption rate of *Aedes albopictus* larvae, *Tubifex* sp. and Optimum fish pellets by four different fishes at the 5% level.

Name of Fish	Consumption Rate (Mean ± SD)		
	***Aedes albopictus* Larvae**	***Tubifex* sp.**	**Optimum Fish pellets**
Carassius auratus (Gold Fish)	49± 2.06	45± 4.38	18.8 ± 4.51
Betta splendens (Betta fish)	46.3± 5.39	38.8 ± 4.01	2 ± 3.06
Poecilia sphenops (Molly fish)	41.8 ± 5.37	39.3 ± 4.98	11 ± 4.03
Poecilia reticulata (Guppy)	38.1 ± 3.75	20± 4.03	1.2 ± 2.41

Table-5: Two way ANOVA of the different fishes on consumption of *Aedes albopictus* larvae, *Tubifex sp.* and Optimum fish pellets

	Sum of Squares	**df**	**Mean Square**	**F value**	**P value**	**Significant**
Fish	4871	3	1624	399.6	P<0.0001	Yes
Aedes aegypti larvae , *Tubifex* sp. and Optimum fish pellets	27824	2	13912	2516	P<0.0001	Yes
Interaction	1477	6	246.2	60.59	P<0.0001	Yes

*Significant at 0.05 level confidence

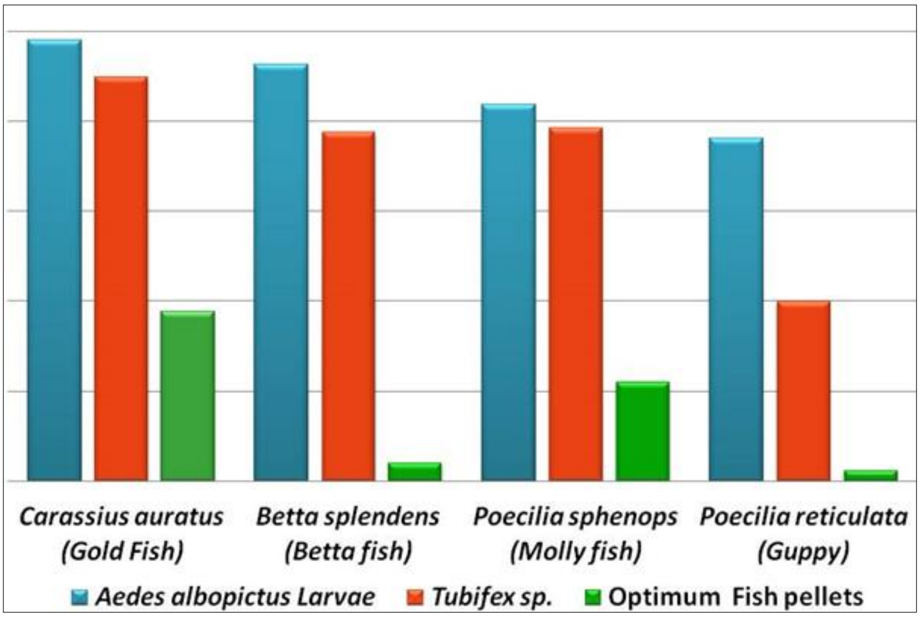

Fig 6: Food preference of four different types of fish for three different types of food items when supplied heterogeneously.

In the third set of experiment, the comparison of the mean consumption rate of *Ae. aegypti* and *Ae. albopictus* of four fishes showed almost no variation and the consumption rate for both the types of larvae was almost at par with each other for all the four fish species. However, the data indicated that rate of feeding of Goldfish, Black molly and Betta fish were very high, as compared to the Guppy fish.

Table 6: The mean consumption rate of *Aedes aegypti* and *Aedes albopictus* larvae, by four different fishes at the 5% level.

Name of Fish	Consumption Rate (Mean ± SD)	
	Aedes aegypti Larvae	*Aedes albopictus* Larvae
Carassius auratus (Gold Fish)	48.6± 2.65	48.2 ± 3.43
Betta splendens (Betta fish)	44.8± 3.55	45.4 ± 4.35
Poecilia sphenops (Molly fish)	40.2 ± 3.67	40.3 ± 4.03
Poecilia reticulata (Guppy)	37.5 ± 4.26	36.1 ± 5.34

Table 7: Two way ANOVA of the different fishes on consumption of *Aedes aegypti* and *Aedes albopictus* larvae

	Sum of Squares	df	Mean Square	F value	P value	Significant
Fish	1581	3	527	96.28	P<0.0001	Yes
Aedes aegypti and *Aedes albopictus* larvae,	1.513	1	1.513	0.5442	P=0.4655	No
Interaction	10.94	3	3.646	1.312	P=0.2855	No

*Significant at 0.05 level confidence

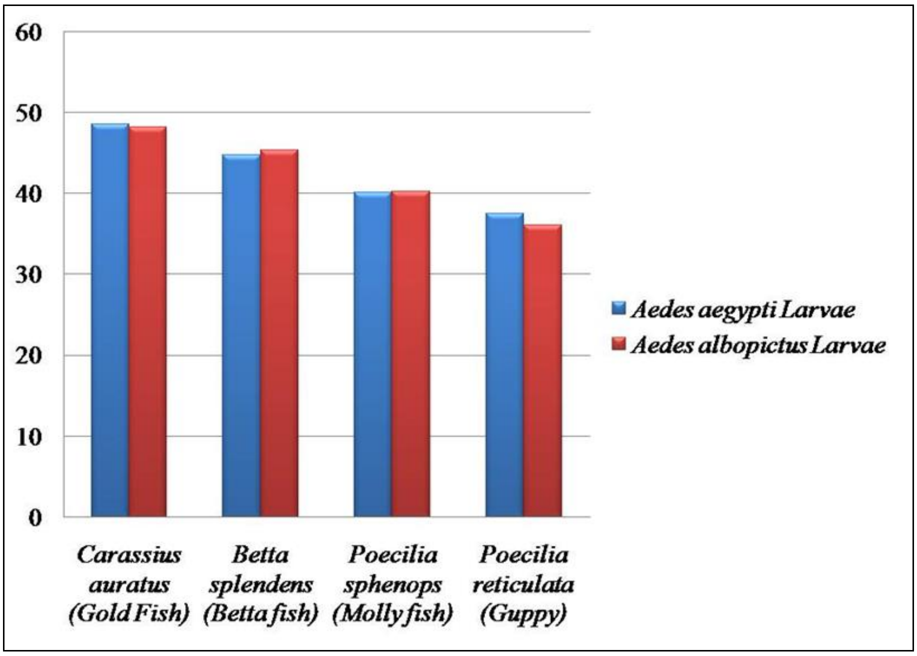

Fig 7: Comparisons between consumption of *Aedes aegypti* and *Aedes albopictus* by four different types of fish

Discussion

In this present study we evaluate the potentiality of these four ornamental fish species. In contrast to *Poecilia reticulata* (Guppy); *Poecilia sphenops* (Black Molly), *Carassius auratus* (Gold Fish), and *Betta splendens* (Betta Fish) all had shown strong larvivorous potentiality. In the laboratory, these fish species are used to reduce the number of mosquito larvae. The present findings are consistent with those of earlier research reports in various ecosystems conducted by Datta *et al*., 2022 [29]; Tilak *et al*., 2007 [26]; Câmara *et al*., 2017 [27]; Sumithra *et al*., 2014 [28] and Gupta *et al*., 2009 [30]. The results of the present study make it clear that all four species of fish prefer live food, particularly mosquito larvae, than artificial food, and that this preference is substantially greater than that of other fish species (Table-3; Table-5; Fig-5; Fig-6). The consumption rates of both types of larvae were near about equal for all four fish species, and the observational data also confirmed this result. Therefore these four ornamental fishes have no particular preference for any of the larvae of the two different species of *Aedes* (Table-7; Fig-7). Production of ornamental fish has become a popular and economically viable industry in several nations throughout the world. Some of these larvivorous fish are also taken directly out of the river for decorative purposes and sold to the nearby aquarium stores, which benefits all parties involved [1]. The widespread distribution of these four fish species further supports their sustainability and ability to adapt to a variety of ecological circumstances [18, 27]. Since the results of a laboratory study have previously been proven, it is anticipated that using the same approach in a natural setting will be successful.

Conclusion

In general, a variety of methods have been used to reduce diseases spread by mosquitoes. These techniques either stop the parasite from growing inside the mosquito or kill the mosquito carrier. However, dependency on chemical vector control strategies, a lack of infrastructure and resources, and inadequate management strategies all reduce the effectiveness of controlling vector-borne diseases. Additionally, environmental alterations and variations in the behaviours of many mosquito species, such as insecticide resistance among mosquito strains and pest resurgence, lead to the failure of chemical insecticide-based mosquito control methods. Even though it is more difficult to use and sustain, bio-control with ornamental fishes is preferable to chemical insecticides. One of its most significant benefits is that it is a method that protects the environment by not introducing toxins.

Therefore, it is recommended to step up research on many facets of fish biology and its function. It is important to adopt strategies like regular monitoring and surveying, identifying breeding areas, and raising public awareness. There is a lack of field research and studies on these fish's effectiveness as prospective larval control agents. Additionally, studies designed to evaluate the viability and effectiveness of these fish species in the wild may be useful in developing vector management strategies to combat *Aedes* mosquito population.

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