International Journal of
Mosquito Research

Anindita Datta
Vector Molecular Genetics
Research Unit, Department of
Zoology (UG & PG), Serampore
College, Serampore, Hooghly,
West Bengal, India

Rishabh Sen
Vector Molecular Genetics
Research Unit, Department of
Zoology (UG & PG), Serampore
College, Serampore, Hooghly,
West Bengal, India

Rittika Banerjee
Vector Molecular Genetics
Research Unit, Department of
Zoology (UG & PG), Serampore
College, Serampore, Hooghly,
West Bengal, India

Pranab Kumar Banerjee
Vector Molecular Genetics
Research Unit, Department of
Zoology (UG & PG), Serampore
College, Serampore, Hooghly,
West Bengal, India

Larvicidal efficacy of ornamental fishes to control *Aedes aegypti* in West Bengal

Anindita Datta, Rishabh Sen, Rittika Banerjee and Pranab Kumar Banerjee

Abstract
Escalating population of *Aedes aegypti* may lead to cause Dengue. However, controlling their larvae by using conventional techniques like insecticides, mosquito repellents are hazardous for human health and environment. Hence, it is recommended to biologically control the mosquito larvae through ornamental fish as they are eco-friendly and cost effective. In the present study, consumption rate of *Aedes aegypti* larvae and other food preference of six fishes (Angel fish, Betta fish, Gold fish, Red swordtail, Molly fish and Guppy) have been observed and a comparative study has been recorded. Angel fish and Gold fish show good efficacy towards all three types of food whereas Molly fish, Fighter and Guppy exhibit good efficacy towards *Aedes aegypti* larvae than the rest two food items, only Red swordtail showed significant deviation in results. So, in near future these fishes can be considered as effective biological agents for controlling *Aedes aegypti* Larvae.

Keywords: *Aedes aegypti*, ornamental fish, biological control, food preference, larvicidial efficacy, biological agents.

1. Introduction
Vector-borne diseases continue to pose a severe problem in tropical and subtropical regions. Mosquitoes can transmit a wide range of life threatening and deabiliting diseases of human, including dengue, yellow fever, malaria, filariasis, and a variety of other diseases, which can cause major health problems and even death. A viral disease transmitted by mosquitoes is dengue fever and its control is one of the country's most difficult tasks, particularly in West Bengal [1]. Each year, 100 to 400 million infections are estimated worldwide by the World Health Organization [2]. To reduce the severity of diseases or respond to dengue outbreaks, the control of the dengue vector can be targeted against either the aquatic larval instars or the adult female mosquito stages of the mosquito [3, 4]. According to a study [5], biotic interactions like competition and predation have the ability to reduce mosquito populations by decreasing the number of mosquito larvae. Introducing or modifying an organism to reduce the population of vectors is known as biological control [6]. Since the early 1900s, mosquito larvae are all preyed upon by a variety of organisms that act as biological control agents, including fish, frog tadpoles, dragonfly larvae, aquatic bugs, and cyclopoid copepods [7]. Comparative research on the feeding habits of introduced and native larvivorous fish is notably uncommon, despite the importance of determining the effects of their introduction on ecosystems [8]. Fish with small sizes that can survive in shallow water and those that are prolific breeders with short life cycles are the ideal candidates to act as biocontrol agents against mosquito larvae. They are surface-feeding predators that prefer mosquito larvae and have no negative effects on the local fish population [9]. Various lines of data [10, 11] revealed that in mosquito control, various mosquito breeding habitats have been exploited with larvivorous fish like *Poecilia reticulata* and *Gambusia affinis*. However, there are few reports of tactical level scaling up or large-scale fish utilisation [12, 13, 14]. In order to efficiently combat dengue vectors, larvivorous fish are mostly recommended by the WHO [15, 16]. However, their effectiveness in preventing or controlling dengue is likely to differ from that of malaria.

Corresponding Author:
Anindita Datta
Vector Molecular Genetics
Research Unit, Department of
Zoology (UG & PG), Serampore
College, Serampore, Hooghly,
West Bengal, India

According to Singh and Ahmed (2005) [17], ornamental fish are the most widely kept creatures in the world today because they are beautiful, colourful varieties of fish with calming nature. Many researchers [18, 19, 20] have reported about the larvivorous capabilities of local fishes. These ornamental fish, on the other hand, have a great potentiality for use in decorative tanks, where they can provide both aesthetic pleasure and mosquito control. In this method, ornamental fish should be introduced to actively prevent mosquito breeding. Tilak *et al.* (2007) [21] pointed out that Goldfish and Blue Gourami have been reported to be efficient larvivorous fishes, akin to Gambusia. Brazilian fish species also exhibited larvivorous capability, with lower biodiversity consequences [22]. Ornamental fishes like *Betta splendens* (Betta fish), *Poecilia reticulata* (Guppy), *Xiphophorus hellerii* (Red swordtail), *Poecilia sphenops* (Black Molly), *Carassius auratus* (Gold Fish), *Pterophyllum scalare* (Angel Fish) are known predators of *Aedes aegypti* larvae. Bettas are slow-moving, placid fish which do not need as much space as other fish. A normal guppy is a prolific breeder who may consume nearly their entire body weight in mosquito larvae on a daily basis. They can quickly and easily move around the pond and find larvae in even the most inconspicuous regions because to their small size. Swordtails are omnivores who eat a wide range of invertebrates, insects and algae. Whereas black Molly is widely occurring viviparous fish that live in fresh or brackish warm environments and are frequently vividly coloured [6]. Smaller goldfish are better at swimming around the pond and most of the mosquito larvae are readily consumed by them. On the other hand Angel fish have spherical bodies, are heavily compressed laterally, and are inherently carnivorous. A very little research work has been done for evaluating the potentiality of ornamental fishes for mosquito larvae control. Thus the main objective is to evaluate the larvicidial efficacy against *Aedes aegypti* larvae of these fish species in the presence of *Tubifex* sp. and fish pellets as alternative food. Therefore, in order to decrease the *Aedes aegypti* population, our study has been conducted to determine the effectiveness of ornamental fishes as predators of *Aedes aegypti* larvae.

2. Materials and methods
2.1 Sites of Collection

Fig 1: Map showing the collection sites of study area

2.2 Description of collection sites

Table 1: Description of collection sites

District	Site of Collections	Latitude	Longitude
Kolkata	Hazra	22.5228°N	88.3500°E
	Tollygunge	22.4986°N	88.3454°E
	Behala	22.5016°N	88.3209°E
Howrah	Shibpur	22.5713°N	88.3109°E
	Salkia	22.6013°N	88.3313°E
Hooghly	Uttarpara	22.6701°N	88.3355°E
	Serampore	22.7505°N	88.3406°E
North 24 Paragana	Dumdum	22.6420°N	88.4312°E
	Dunlop	22.6519°N	88.3786°E
	Barasat	22.7248°N	88.4789°E
South 24 Paragana	Diamond Harbour	22.1927°N	88.1895°E

2.3 Collection of Larvae

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


Fig 2: *Aedes aegypti* larva

2.4 Collection of Fish, Fish pellets and Tubifex

Pterophyllum scalare (Angelfish) of 8cm length, *Carassius auratus* (Goldfish) of 6.2 cm length, *Poecilia reticulata* (Guppy) 3 cm length, *Betta splendens* (Betta fish) of 7.1 cm length, *Xiphophorus hellerii* (Swordtail) of 4.3 cm length and *Poecilia sphenops* (Black molly) of 2.7 cm length and of same age group were bought from ornamental fish shops of Kolkata and Dumdum. Artificial fish pellets (Tokyu) and live *Tubifex* sp. were collected from local aquarium shops in Kolkata. Fishes were separated in six 1L beakers and kept in the laboratory condition for one week (Fig.2). *Tubifex* sp. were kept in plastic tray submerged in water.
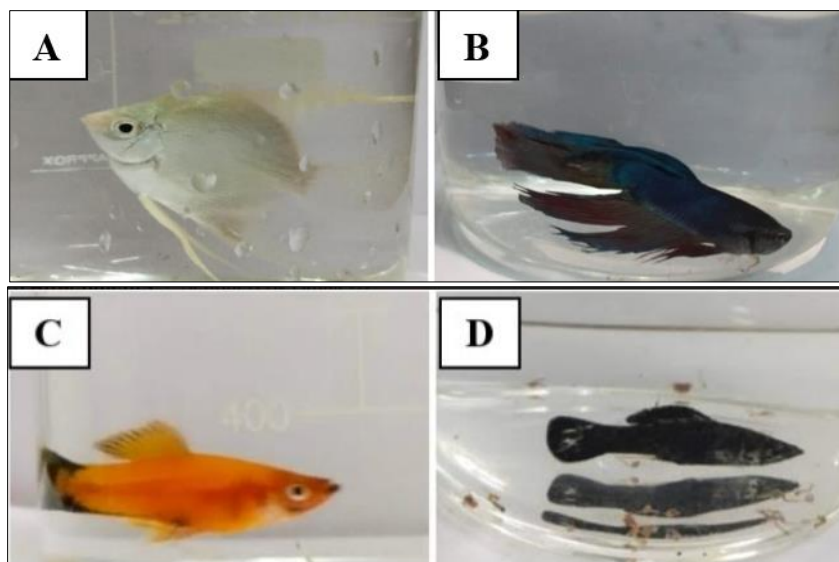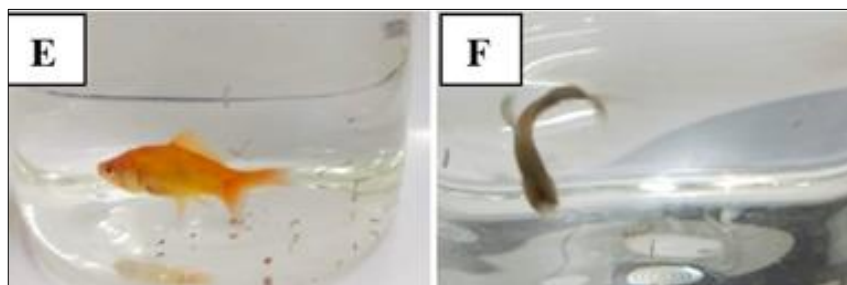
[A] *Pterophyllum scalare* (Angel Fish), [B] *Betta splendens* (Betta fish), [C] *Xiphophorus hellerii* (Red swordtail),
[D] *Poecilia phenops* (Black Molly), [E] *Carassius auratus* (Gold Fish), [F] *Poecilia reticulata* (Guppy)

Fig 3: Acclimatization period of six fish species in laboratory condition:

2.5 Experimental Design

The fishes were kept without any supply of food for 24 hours to acclimatize in the laboratory condition and to make sure that they were in fasting condition before starting the experiments. Three different sets of experiments with three different sets of food combination were run separately for each of the fish species and the temperature was set 25 °C. Each experiment was done with five replicates to make sure that there is a sufficient time period for the fishes to get used to of the food combinations given before starting a new set of combination, so that the larvicidal activity towards the *Aedes aegypti* larvae could be observed in a more profound and prominent way.

In the first set of experiment, each fish was supplied with twenty (20) *Aedes* larvae of all instar stages and twenty (20) Tokyu fish pellets and were observed for 2 hours (Fig.3). In the second set, the combination of food was changed to *Aedes aegypti* larvae with *Tubifex* sp. Each fish was given 20 larvae of different instar stages with 20 *Tubifex* sp. and were observed for 2 hours (Fig.4). In case of the third set of experiment, *Aedes aegypti* larvae, *Tubifex* sp. and Tokyu fish pellets were supplied together. Each fish was supplied with 13 *Aedes aegypti* larvae of different instar stages, 14 *Tubifex* sp. and 13 Tokyu fish pellets, and were observed for 2 hours (Fig.5). The amount of ingested larvae and the other two food options were recorded.
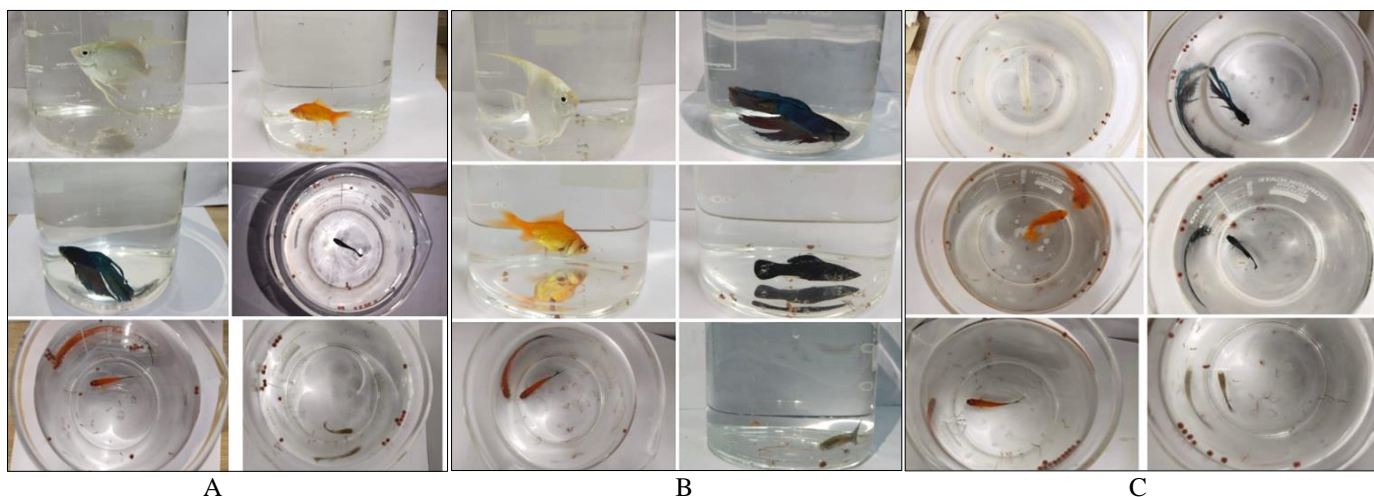
Fig 4: (A) *Aedes aegypti* larvae and Tokyu fish pellets are supplied to each fish; (B) *Aedes aegypti* larvae and Tubifex are supplied to each fish;
(C) *Aedes aegypti* larvae, Tokyu fish pellets and Tubifex are supplied to each fish

2.6 Statistical Analysis

For each food combination, two-way ANOVA followed by Tukey's multiple comparison test was run for six fish species and combination of food for five days of observation using Graphpad Prism (Version-8). Statistical differences were set at $p<0.05$.

3. Results & Discussion
3.1 Results

In the first experiment conducted to know about the food preference of six fishes between *Aedes aegypti* larvae and fish pellets, all the fishes always showed significantly ($P<0.001$) higher preference for mosquito larvae than artificial fish pellets. Amongst all the six species, Guppy showed higher preference for I and II instar larvae of *Aedes aegypti* whereas rest of the fishes devoured all instar larvae (Table-2), (Fig.5). The two way ANOVA result showed the interaction is extremely significant at 0.05 level confidence. (Table-3)

Table 2: The mean consumption rate of *Aedes aegypti* larvae .and Tokyu fish pellets by six different fishes at the 5% level

Name of Fish	Consumption Rate (Mean ± SD)	
	Aedes aegypti **Larvae**	**Tokyu Fish pellets**
Pterophyllum scalare(Angel Fish)	20 ± 0	20 ± 0
Betta splendens (Betta fish)	20 ± 0	1 ± 1.38
Carassius auratus (Gold Fish)	20 ± 0	19 ± 3.39
Xiphophorus hellerii(Red swordtail)	20 ± 0	0.8 ± 1.64
Poecilia sphenops(Molly fish)	20 ± 0	14.2 ± 3.77
Poecilia reticulata (Guppy)	13.4 ± 5.98	0.4 ± 1.07

Table 3: Two-way ANOVA of the six different fishes on consumption of *Aedes aegypti* larvae and Tokyu fish pellets

	Sum of Squares	df	Mean Square	F value	P value	Significant
Fishes	1515	5	302.9	206.5	P<0.0001	Yes
Aedes aegypti larvae and Tokyu fish pellets	1402	1	1402	966.7	P<0.0001	Yes
Interaction	931.5	5	186.3	128.5	P<0.0001	Yes

*Significant at 0.05 level confidence
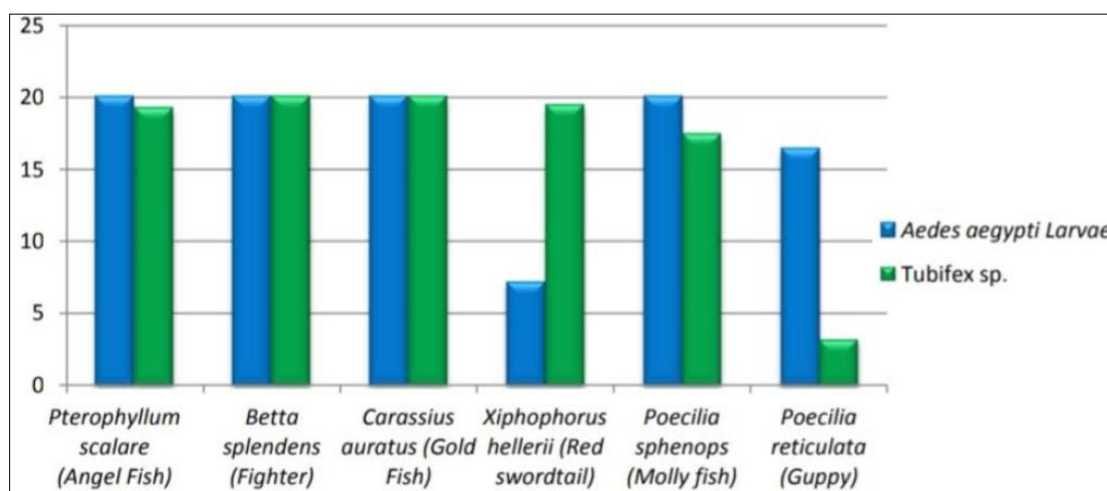
Fig 5: Food preference of six different types of fish for two different types of food items when supplied heterogeneously.

In the second experiment when two live foods, *Aedes aegypti* larvae and *Tubifex* sp. were supplied together, Angle fish ,Gold fish and Betta preferred both types of food (p < 0.001), whereas red swordtail preferred *Tubifex* sp. more than mosquito larvae. Molly and Guppy both showed significantly higher preference for *Aedes aegypti* larvae (p < 0.001) but Guppy, although preferred I and II instar larvae, the preference for mosquito larvae was significantly higher than that of Molly (Table-4), (Fig.6). The two way ANOVA result showed the interaction is extremely significant at at 0.05 level confidence (Table-5).

Table 4: The mean consumption rate of *Aedes aegypti* larvae .and *Tubifex* sp.by six different fishes at the 5% level

Name of Fish	Consumption Rate (Mean ± SD)	
	Aedes aegypti **Larvae**	*Tubifex* **sp.**
Pterophyllum scalare (Angel Fish)	20 ± 0	19.2 ± 1.64
Beta splendens (Betta fish)	20 ± 0	20 ± 0
Carassius auratus (Gold Fish)	20 ± 0	20 ± 0
Xiphophorus hellerii (Red swordtail)	7.2 ± 3.77	19.4 ± 1.75
Poecilia sphenops (Molly fish)	20 ± 0	17.4 ± 5.11
Poecilia reticulata (Guppy)	16.4 ± 5.97	3.2 ± 3.77

Table 5: Two way ANOVA of the six different fishes on consumption of *Aedes aegypti* larvae and *Tubifex* sp.

	Sum of Squares	df	Mean Square	F value	P value	Significant
Fish	931.2	5	186.2	184.7	P<0.0001	Yes
Aedes aegypti larvae and *Tubifex* sp.	8.067	1	8.067	2.554	P=0.1231	No
Interaction	818.1	5	163.6	51.81	P<0.0001	Yes

*Significant at 0.05 level confidence
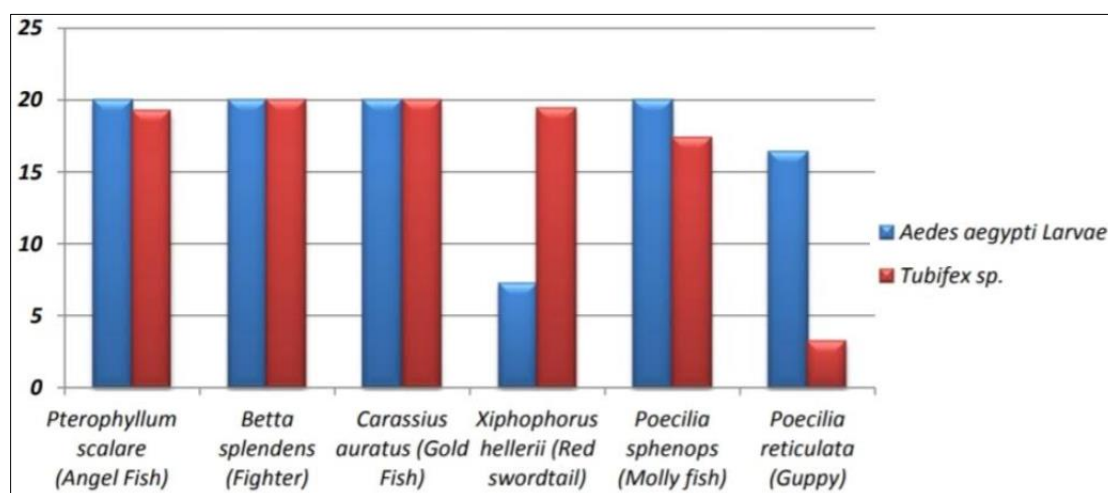
Fig 6: Food preference of six different types of fish for two different types of food items when supplied heterogeneously.

In the third experiment when all the three types of food items, *Aedes aegypti* larvae, *Tubifex* sp. and the fish pellets were supplied together to each species of fish, all of them except red swordtail showed significantly ($p<0.001$) higher preference for *Aedes aegypti* larvae. It has been observed (Table-6), (Fig.7) that Angel and Gold fish consumed almost all three types of food within the given time limit whereas Betta, Molly and Guppy preferred *Aedes* larvae more than the rest two food items. The two way ANOVA result showed the interaction is extremely significant at at 0.05 level confidence. (Table-7)

Table 6: The mean consumption rate of *Aedes aegypti* larvae, *Tubifex* sp. and Tokyu fish pellets by six different fishes at the 5% level.

Name of Fish	Consumption Rate (Mean ± SD)		
	Aedes aegypti Larvae	*Tubifex* sp.	Tokyu Fish pellets
Pterophyllum scalare (Angel Fish)	13 ± 0	14 ± 0	11.8 ± 2.55
Betta splendens (Betta fish)	13 ± 0	14 ± 0	0 ± 0
Carassius auratus (Gold Fish)	13 ± 0	14 ± 0	13 ± 0
Xiphophorus hellerii (Red swordtail)	5.4 ± 8.61	13.8 ± 0.88	0 ± 0
Poecilia sphenops (Molly fish)	13 ± 0	14 ± 0	5 ± 3.09
Poecilia reticulata (Guppy)	7.4 ± 4.51	3.2 ± 3.22	0 ± 0

Table 7: Two way anova of the six different fishes on consumption of *Aedes aegypti* larvae, *Tubifex sp.* and Tokyu fish pellets

	Sum of Squares	df	Mean Square	F value	P value	Significant
Fish	1096	5	219.3	140.0	*P*<0.0001	Yes
Aedes aegypti larvae, *Tubifex* sp. and Tokyu fish pellets	877.4	2	438.7	236.1	*P*<0.0001	Yes
Interaction	612.8	10	61.28	32.97	*P*<0.0001	Yes

*Significant at 0.05 level confidence
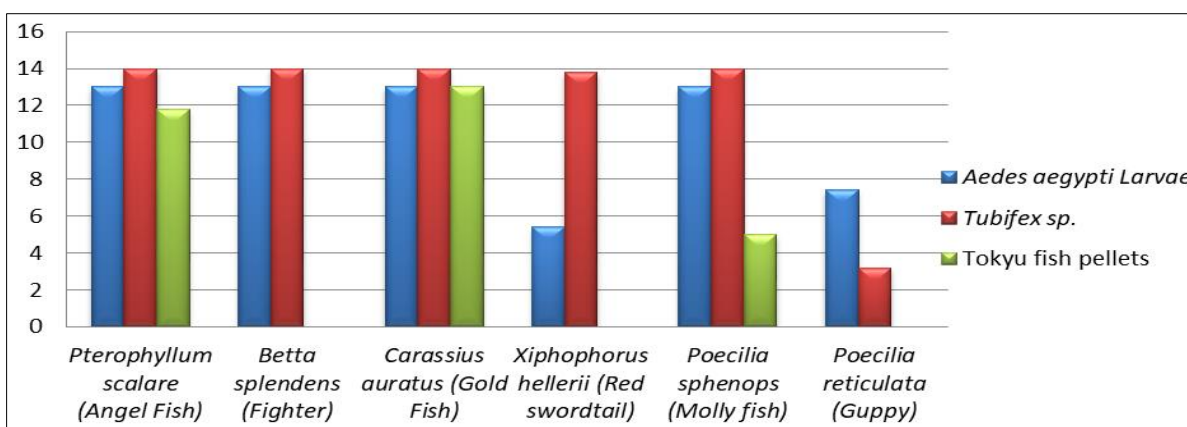
Fig 7: Food preference of six different types of fish for three different types of food items when supplied heterogeneously.

3.2 Discussion
The findings of the present study make it clear that all fish prefer live food to artificial food by a significant margin. The relative frequency of alternative prey can also modify the consumption rate of mosquito larvae of the fishes [23]. In our findings the presence of *Tubifex* sp. as alternative prey has considerably influenced the *Aedes aegypti* larvae consumption rate of all the six fishes (Angel fish, Gold fish, Red Swordtail,

Guppy, Black Molly and Betta). Larvivorous fishes have been shown to be excellent biological mosquito controllers in stable water bodies with high water quality [24]. However, stagnant water with very little oxygen is the main cause of the overpopulation of *Aedes* mosquito. As a result, the three basic criteria for a fish to be utilized as a mosquito bio control agent are: first, it must have high predation efficacy on mosquito larvae; second, it must be resistant to low oxygen concentrations while having high tolerance to toxic metabolites [25]. Earlier works [9] have considered gold fish showed good larivorous activity towards mosquito larvae and it has high fecundity [26]. Luciano *et al.*, (2007) [27] showed that the male *Poecilia reticulata* can feed on large amount of larvae, compared to its female counterpart and they also highlighted that Each day, *Betta splendens* may eat 523 larvae per gram of body weight. Therefore, compared to a single individual, a large group can consume more larvae. Martinez *et al.*, (2002) [28] suggested that ten *Poecilia sphenops* specimen be used in each artificial domestic container for effective control of *Aedes* larvae. Câmara *et al.*, (2017) [22] suggested that use of angelfish as controlling agent of *Aedes* larvae should be encouraged, since they can be effective against these deadly vectors of Dengue Hemorrhagic Fever (DHF). 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.

4. Conclusion

According to the aforementioned study, it is possible to conclude that the utilisation of ornamental fish can be helpful to control *Aedes aegypti* larvae. Although each species of fish, except red swordtail showed significantly higher preference for *Aedes* larvae (Table 3, Fig. 7; Table 5, Fig.8) but all of the six fishes (Angel fish, Gold fish, Red Swordtail, Guppy, Black Molly and Betta) can be utilised successfully as a potent bio-control agent for control of *Aedes aegypti* larvae to prevent the spread of diseases including dengue, chikungunya, yellow fever, and others as well as to control mosquitoes that have developed or may develop chemical resistance. As the ornamental fishes are habituated mostly in artificial habitat, so the viability of these fishes in natural environment is considerably less. Therefore, the application of ornamental fishes ought to be considered in control of mosquito larvae population when other ways fail.

5. Acknowledgement

6. References

1. Majhi J, Singh R, Yadav V, Garg V, Sengupta P, Atul PK, *et al*. Dynamics of dengue outbreaks in gangetic West Bengal: A trend and time series analysis. Journal of family medicine and primary care. 2020;9(11):5622-5628.https://doi.org/10.4103/jfmpc.jfmpc_800_20
2. World Health Organization. Dengue and Severe Dengue. Factsheet, revised March 2020. Geneva: World Health Organization, 2020.
3. World Health Organization. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control, World Health Organization, 2009. ISBN 978 92 4 154787 1; http://whqlibdoc. who.int/publications/2009/9789241547871_eng.pdf.
4. World Health Organization. Global Strategy for Dengue Prevention and Control. (in IRIS). World Health Organization: Geneva, 2012-20.
5. Knight TM, Chase JM, Goss GM, Knight JJ. Effects of interspecific competition, predation, and their interaction on survival and development time of immature Anopheles quadrimaculatus. J. Vector Ecol. 2004;29(2):277-284.
6. Sumithra V, Janakiraman A, Altaff K. Bio-Control of Mosquito Larvae Through The Black Molly, Poecilia Sphenops. International Journal of Pure and Applied Zoology, 2014, 2.
7. Raghavendra K, Subbarao SK. Chemical insecticides in malaria vector control in India. ICMR bull. 2002;32(10):1-7.
8. Hoddle MS. Restoring balance: using exotic species to control invasive exotic species. Consery. Biol. 2004; 18(1):38-49.
9. Chandra G, Bhattacharjee I, Chatterjee SN, Ghosh A. Mosquito control by larvivorous fish. Indian J Med Res. 2008; Jan;127(1):13-27. PMID: 18316849.
10. 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):1-8.
11. Ghosh SK, Chakaravarthy P, Panch SR, Krishnappa P, Tiwari S. Comparative efficacy of two poeciliid fish in indoor cement tanks against chikungunya vector *Aedes aegypti* in villages in Karnataka, India. BMC Public Health. 2011;11(1):599.
12. Ghosh SK, Tiwari SN, Kulshreshtha AK, Sathyanarayan TS, Sampat TRR. Control of malaria transmission using larvivorous fishes. In: Trends in Malaria and Vaccine Research-the Current Indian Scenario. (Eds. D. Raghunath and R. Nayak). Tata McGraw-Hill Publishing Company Ltd., New Delhi, 2002, 154-158.
13. Ghosh SK, Tiwari SN, Sathyanarayan TS, Sampath TR, Sharma VP. Larvivorous fish in wells target the malaria vector sibling species of the Anopheles culicifacies complex in villages in Karnataka, India. Trans R. Soc. Trop. Med. Hyg. 2005;99(2):101-105.
14. Haq S, Yadav RS, Kohli VK. Developing larvivorous fish network for mosquito in urban areas: a case study. ICMR Bull. 2003;33(7):69-73.
15. WHO. Report of the travelling seminar on the use of larvivorous fish for mosquito control in anti-malaria campaigns. Golden Sands Bulgaria/Tbilisi Georgian Republic, SSR, USSR. 1980 (1979); EM/VBC/22: EM/TR.SMR. LRV.FSH.MSQ.CTR/14, 35 pp.
16. WHO. Manual on environmental management for mosquito control with special emphasis on malaria vector. World Health Organization, 1982; Publ. No. 66, Geneva, 283 pp.
17. Singh AK, Ahmed SH. Ornamental Fish Culture in Bihar: Prospects as a Cottage Industry. Fishing Chimes. 2005;25(6):9-18
18. Milam CD, Farris JL, Wilhide JD. Evaluating mosquito control pesticides for effect on target and non-target organisms. Arch. Environ. Contam. Toxicol., 2000;39(3):324-328.
19. Alshaimaa MR Hamed, Mona S El-Sherbini, Magda SA Abdeltawab. Eco-Friendly Mosquito-Control Strategies:

Advantages and Disadvantages. Egypt. Acad. J Biolog. Sci. E-Medical Entom. & Parasitology. 2022;14(1):17-31 DOI: 10.21608/EAJBSE.2022.221601

20. Pahari PR, Mishra NP, Sahoo A, Bhattacharya RP, Bhattacharya T. First record of the mosquito control potentiality of *Stigmatogobius sadanundio* (F. Hamilton, 1822) Gobiidae, Perciformesin laboratory condition. Tropical parasitology, 2020;10(2):130-135. https://doi.org/10.4103/tp.TP_55_19

21. Tilak Rina, Dutta J, Gupta K. Prospects for the use of ornamental fishes for mosquito control: A laboratory investigation. Indian journal of public health. 2007;51(1):54-5.

22. Câmara Miraldo, Marcel, Pecora, Iracy. Efficiency of Brazilian native ornamental fishes as mosquito larvae predators. Boletim do Instituto de Pesca, 2017;43:93-98. https://doi.org/10.20950/1678-2305.2017.93.98.

23. Quintans F, Scasso F, Defeo O. Unsuitability of *Cnesterodon decemmaculatus* (Jenyns, 1842) for mosquito control in Uruguay: Evidence from food-preference experiments. J Vector Ecol, 2010;35(2):333-338

24. Ahmed YA, Isfaq A. Field trial on the impact of *Oreochromis spilurus spilurus* on malaria transmission in northern Somalia. W.H.O. 1982; EM/MAL/191 EM/SOM/MPD/OO1/ RB, 25 pp.

25. Pant CP, Rishikesh N, Bang YH, Smith A. Progress in malaria vector control. Bull. W.H.O. 1981;59:325-333.

26. Kuznetsov VA. Changes in the population structure and biological indices of the goldfish *Carassius auratus gibelio* in the Volga Stretch of the Kuibyshev Reservoir under conditions of intense anthropogenic load on the ecosystem. Journal of Ichthyology, 2004;44(2):167-174.

27. Luciano P de GC, Ricardo JSP, Ana CFR, Francisco J de PJ, Rodrigo LF, Emanuel PS, *et al*. Efficacy of fish as predators of *Aedes aegypti* larvae, under laboratory condition. Rev. Saude Publica. 2007;41(4):1-6.

28. Martinez-Ibara JA, Guilen YG, Arredondo-Jimenes JI, Rodriguez-Lopes MH. Indigenous fish species for the control of *Aedes aegypti* in water storage tanks in Southern Mexico. Bio Control. 2002;47(4):481-486.