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Assessment of larval feeding efficiency of ornamental fishes for control of *Aedes* and *Culex* mosquitoes

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Abstract
Current mosquito control strategies heavily rely on the use of non-specific insecticides, leading to adverse environmental effects. Therefore, adopting a more specific and biological approach, such as use of larvivorous fish for control of mosquito larvae, proves to be a sustainable alternative. The objective of this study is to find out the larvivory potential of five ornamental fish species namely *Puntius tetrazona*, *Gymnocorymbus ternatzi*, *Danio rerio*, *Pethia conchonius* and *Poecilia sphenops*. Among the studied species, *Puntius tetrazona* demonstrated a significantly higher mean consumption rate of mosquito larvae, indicating its pronounced larvivory potential. However, all the fish species showed a significant difference of consumption rate among different feed types. These results highlight the importance of these ornamental fishes in controlling mosquito populations, emphasizing their potential role in mosquito-borne disease management strategies.

Keywords: Ornamental fishes, mosquito control, *Aedes albopictus*, *Culex quinquefasciatus*, biological control, larvivory potential

1. Introduction

Mosquitoes are vectors for numerous debilitating and potentially fatal diseases, including malaria, dengue fever, zika, and chikungunya. Traditional vector control methods mostly rely on chemical insecticides, which are harmful to the environment and might result in the emergence of insecticide resistance. In recent years, there has been an increase in interest in exploring more natural and sustainable approaches to mosquito management. Although the concept of utilizing fishes for biological control is not a novel idea, its effectiveness has been demonstrated in different locations on multiple occasions. Regrettably, as time has passed, this approach has been neglected and largely forgotten [1, 2]. Among the live feeds taken by fish, mosquito larvae are regarded as a highly desired food source. This is true especially for larvivorous fish species that naturally prefer to hunt on mosquitoes in their immature stages [3]. The application of biological control, particularly the use of larvivorous fish, proved to be essential for malaria control programmes in the 20th century. This strategy was especially important in urban and peri-urban regions since it offered quick fixes that worked for both developed and developing countries [4]. In order to ensure efficient mosquito control, larvivorous fish must possess specific attributes. These fish should exhibit characteristics such as diminutive size, resilience, and the ability to navigate effortlessly in shallow waters containing dense vegetation, which serve as ideal breeding grounds for mosquitoes [5]. Prior to the 1940s, in order to establish efficient management of mosquito populations, numerous nations have implemented the introduction of larvivorous fish, such as *Gambusia affinis* and *Poecilia reticulata*, following comprehensive assessments of their larvivorous capabilities [6]. Subsequently, only a limited number of studies have been conducted to investigate the potential of ornamental fishes for mosquito larvae control. According to Tilak *et al.*, (2007) [7] the inclusion of ornamental fishes in decorative tanks offers substantial promise, as it serves a dual purpose by enhancing the visual appeal and exerting effective control over mosquito breeding. In a recent study, it was demonstrated that ornamental fish species such as guppy, betta, goldfish, angel, and red swordtail possess notable larvivorous abilities and can be used as effective bio-control agents for managing *Aedes aegypti* larvae [8]. In this work, five ornamental fish species with larvivorous capabilities were selected. The chosen species for this study includes Tiger barb (*Puntius tetrazona*), Widow tetra (*Gymnocorymbus ternatzi*), Zebra fish (*Danio rerio*), Rosy barb (*Pethia conchonius*), and Molly (*Poecilia sphenops*). Among them *Danio rerio* and *Pethia conchonius* are native to India and having ornamental value. The goal of this work is to conduct a comparative evaluation of the larvicidal efficacy against *Aedes albopictus* and *Culex quinquefasciatus* larvae, exhibited by these fish species.

2. Materials and Methods

2.1 Larvae collection from natural habitat

The collection of *Aedes* larvae was carried out from diverse locations within the Darjeeling district, focusing on tyres, tree holes, clay pots, and bamboo stumps. Concurrently, *Culex* larvae were collected from sewage drains of Shivmandir and Siliguri town area (Table 1 and Fig. 1). The larval collection activities were conducted during the period from February to March. Following the larvae collection, one generation was reared in the laboratory to facilitate species identification (Fig. 2).

Table 1: Mosquito larvae sampling sites

Mosquito species	Collection sites	Latitude	Longitude
Aedes albopictus	Matigara	26.7223° N	88.3810° E
	Sukna	26.7891° N	88.3646° E
Culex quinquefasciatus	Shivmandir	26.7096° N	88.3616° E
	Siliguri	26.7324° N	88.4176° E

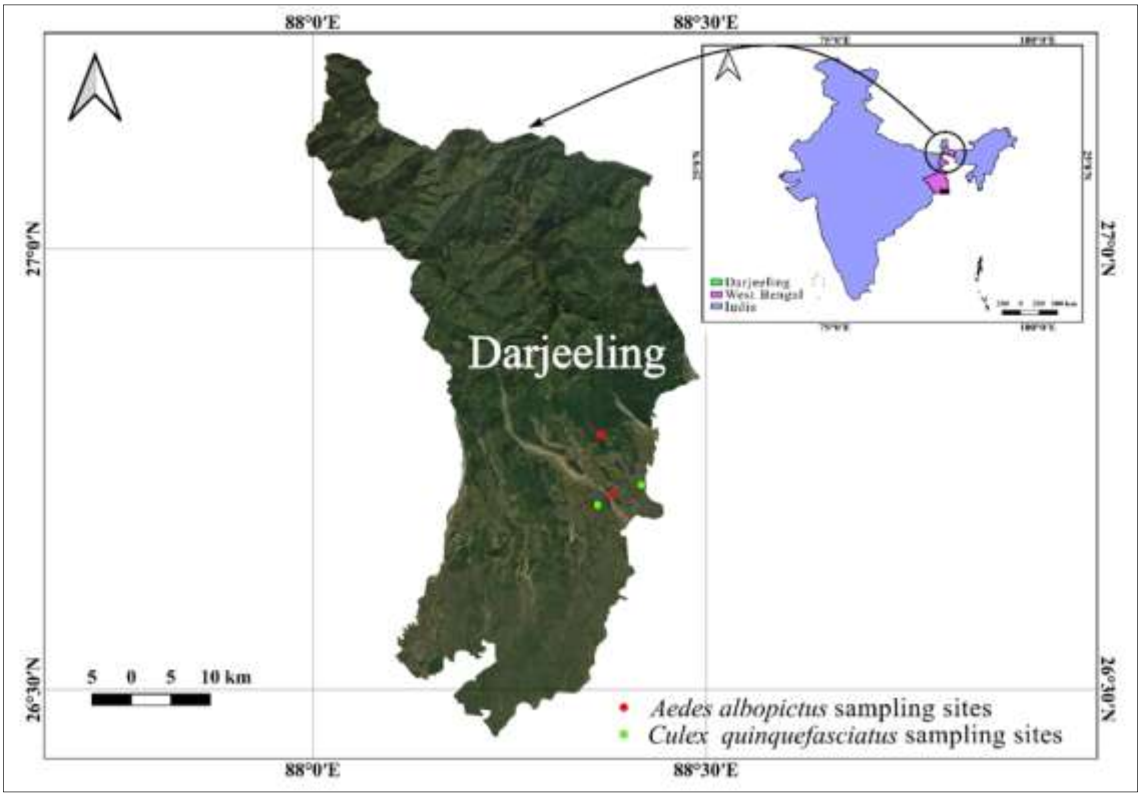

Fig 1: Sampling sites of mosquito larvae. (Map was prepared in QGIS software version 3.30) [17]

Fig 2: Mosquito larvae. (a) *Aedes albopictus* (b) *Culex quinquefasciatus*

2.2 Collection of ornamental fishes
Healthy adult individuals of different ornamental fish species were selected for the study. The ornamental fishes were purchased from several ornamental fish shops in Siliguri. Specifically, three individuals of each of the five distinct fish species were obtained for the experimental purposes. These species included Tiger barb (*Puntius tetrazona),* Widow tetra (*Gymnocorymbus ternatzi*), Zebra fish (*Danio rerio*), Rosy barb (*Pethia conchonius*) and Molly (*Poecilia sphenops*). (Fig.3) Upon purchase, size of each fish was measured (Table 2) and individually housed in 1-liter beaker, maintaining a temperature range of 24±03 °C for a duration of 10 days for acclimatization to reduce any potential stress-related biases. Throughout this period, Tokyu fish pellets were provided as a supplementary food source to the fish.

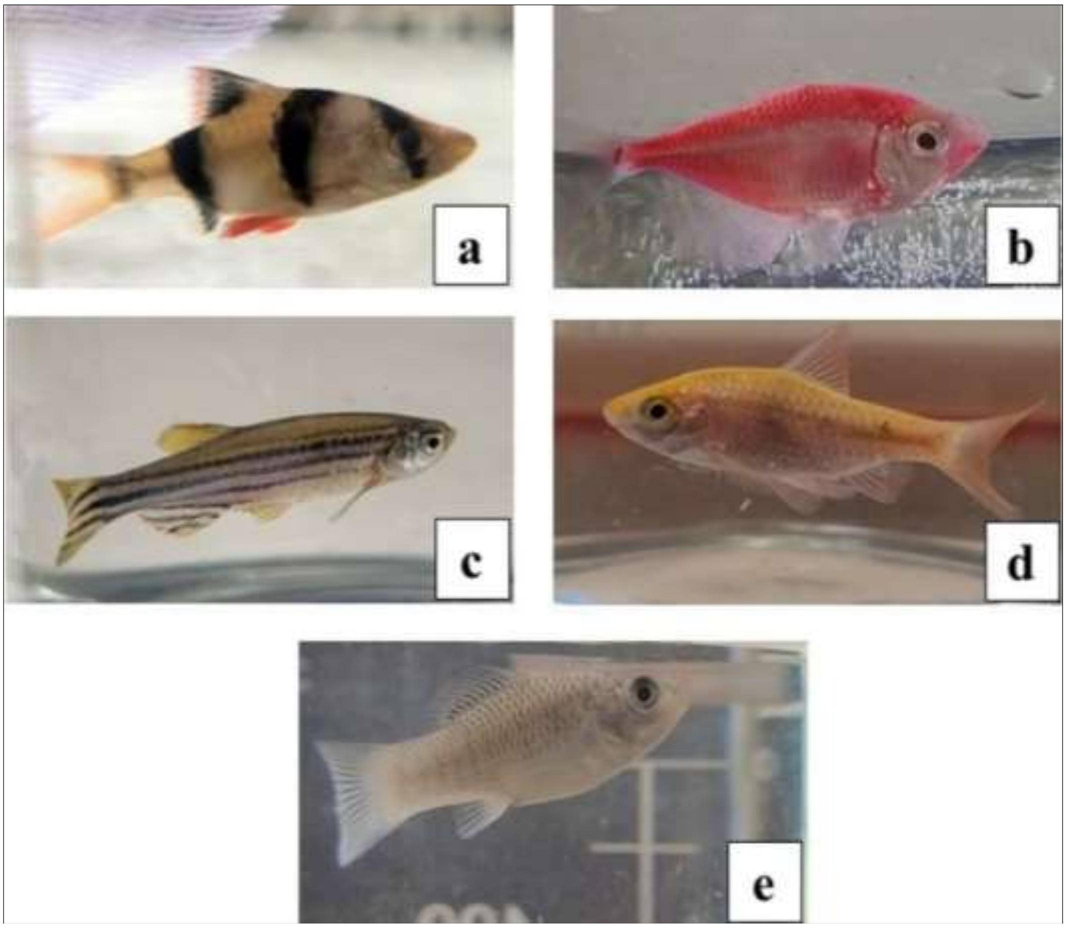

Fig 3: Five ornamental fishes were selected for the experiments. (A) Tiger barb (*Puntius tetrazona*), (B) Widow tetra (*Gymnocorymbus ternatzi*), (C) Zebra fish (*Danio rerio*), (D) Rosy barb (*Pethia conchonius*) (E) Molly (*Poecilia sphenops*)

Table 2: Name of the ornamental fishes used in the experiments along with their family name and size

Serial No.	Fish name	Scientific name	Family	Mean Size (cm) ± SD
01.	Tiger barb	*Puntius tetrazona*	Cyprinidae	4.57±0.21
02.	Widow tetra	*Gymnocorymbus ternatzi*	Characidae	4.43±0.15
03.	Zebra fish	*Danio rerio*	Cyprinidae	3.43±0.25
04.	Rosy barb	*Pethia conchonius*	Cyprinidae	4.27±0.15
05.	Molly	*Poecilia sphenops*	Cyprinodontiformes	4.27±0.21

2.3 Study design
The experiments consisted of two distinct phases. In the first phase, larvivorous potential of each ornamental fish were evaluated. For that, each selected fish underwent a 24-hour starvation period with no access to any food source. After the starvation period, the fish in each tank were supplied with five laboratory-reared late third or early fourth instar larvae of *Aedes albopictus* mosquito. Using a stopwatch, the time taken by each fish to consume the five mosquito larvae was recorded, starting from the introduction of the larvae into the tank until they were completely consumed. The entire experiment was replicated thrice on separate days to ensure the reliability and consistency of the results. Additionally, the same sets of experiments were conducted with larvae of *Culex quinquefasciatus* mosquito, for a comparison between the two mosquito species in terms of the larvivorous potential of the ornamental fish.

In the second phase of the experiment the three individuals of each fish species were supplied with 300 larvae (different instar stages) of *Aedes albopictus* mosquito, 300 larvae (different instar stages) of *Culex quinquefasciatus* mosquito and 300 Tokyu Pellet Fish Food respectively. The feeding rate of each fish was recorded at hourly intervals for three hours to measure their larval feeding efficiency towards different mosquito species and artificial food supplement. This phase of the experiment was also triplicated to ensure data accuracy and reliability.

2.4 Statistical analysis

Two-way ANOVA was performed (using IBM SPSS Statistics 21) to examine the significant differences in the consumption rates of three different feeds across five diverse fish species. The level of statistical significance was established at $p<0.05$.

3. Results & Discussion

3.1 Results

In the first set of experiment, as mentioned earlier, five larvae of *Culex* and *Aedes* were given to each fish to know the larvivorous potential of each fish, and the result shows (Fig.4) Tiger barb, Widow tetra and Zebra fish consumed the *Culex* larvae in less amount of time whereas Rosy barb and Molly consumed the *Aedes* larvae more efficiently. Among all the fish species, the Tiger barb demonstrated a higher larvivorous potential for both *Aedes* (6.22±0.41 sec) and *Culex* sp. (4.08±0.62 sec), with the Widow tetra following closely by consuming *Aedes* (6.52±0.83 sec) and *Culex* (4.32±0.39 sec). On the other hand, Zebra (*Aedes*: 19.59±3.63 and *Culex*: 12.56±2.80), Rosy barb (*Aedes*: 9.68±2.75 and *Culex*: 16.45±0.98) and Molly (*Aedes*: 16.10±3.46 and *Culex*: 26.26±2.16) takes more time to consume the larvae of different mosquito species.

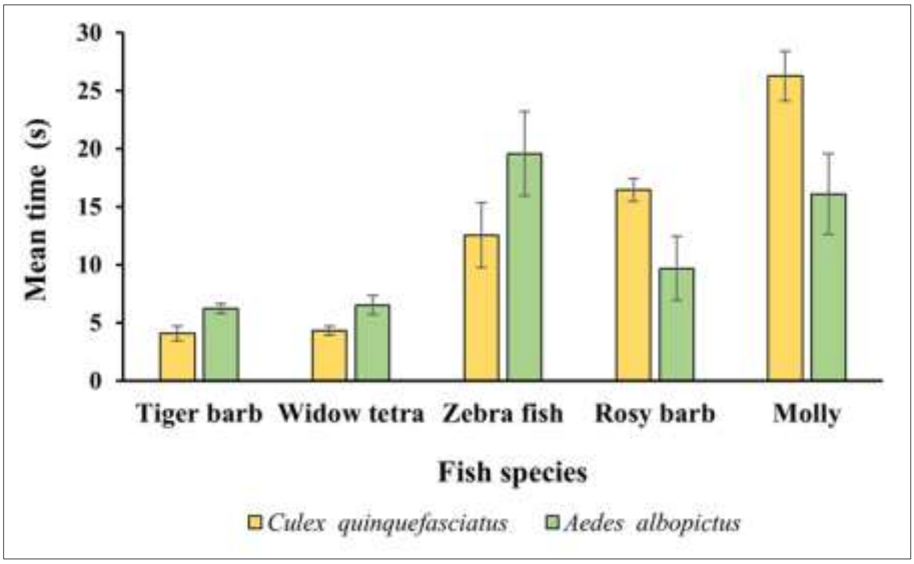

Fig 4: The consumption time of *Aedes* and *Culex* larvae by different ornamental fishes (data represented here is mean time±SE)

As per the results the hourly feeding rates (Table 3 and Fig. 5) of each fish in relation to three distinct feed types showed that Tiger barb consumed more mosquito larvae (both *Culex* and *Aedes*) followed by Widow tetra, Zebra fish, Rosy barb and Molly. The consumption rate for commercially available fish food (Tokyu pellet fish food) showed that Tiger barb and Rosy barb consumed more pellets as compared to other fish species. Furthermore, it was observed that all fish species consumed a greater quantity of live feed in comparison to artificial food pellets. Among the two mosquito species all the fish prefers *Aedes albopictus* over *Culex quinquefasciatus* larvae. The statistical analysis of the one-hour consumption rate using a two-way ANOVA exhibited highly significant results at a 5% confidence level, as shown in table 5.

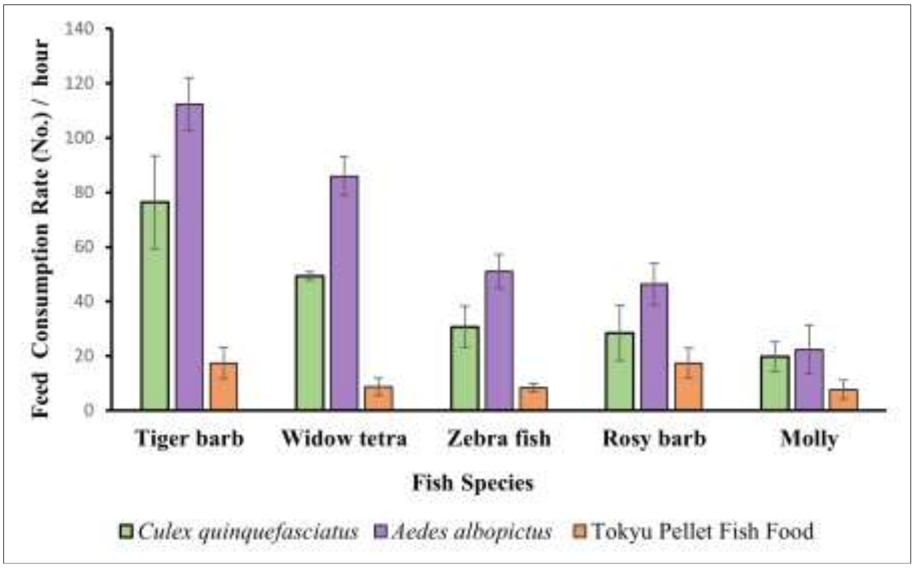

Fig 5: Consumption rate (number/ hour) for three different feeds among five fish species. (Data represented here is Mean±SD)

The data obtained from the three hours experiment shows slightly variation from the hourly consumption rate. Three hours consumption rate for *Culex quinquefasciatus* larvae was higher in Tiger barb followed by Widow tetra, Rosy barb, Molly and Zebra fish. But in case of *Aedes albopictus* larvae, the consumption rate of Molly was slightly lesser than other fish species (Table 4 and Fig. 6). Except molly all the fish species has shown the preference for *Aedes albopictus* in comparison to *Culex quinquefasciatus* larvae. Moreover, all the fish species has consumed near about similar number of artificial fish pellets. The two-way ANOVA for the three-hour consumption rate also demonstrated highly significant results at a 5% confidence level, as shown in table 6.

Table 3: The hourly feeding rates of various ornamental fish species in relation to three distinct feed types

Fish species	Consumed feed (number) / hour (Mean±SD)		
	Culex quinquefasciatus	*Aedes albopictus*	Tokyu Pellet Fish Food
Tiger barb	76.33±17.01	112.33±9.71	17.33±5.69
Widow tetra	49.33±1.53	86.00±7.21	8.67±3.06
Zebra fish	30.67±7.51	51.00±6.25	8.33±1.53
Rosy barb	28.33±10.07	46.33±7.64	17.33±5.51
Molly	19.67±5.51	22.33±9.02	7.67±3.51

Table 4: Feeding rates of different ornamental fishes in three hours with respect to different feed types

Fish species	Consumed feed (number) in three hours (Mean±SD)		
	Culex quinquefasciatus	*Aedes albopictus*	Tokyu Pellet Fish Food
Tiger barb	83.33±10.50	176.00±14.11	29.00±04.58
Widow tetra	63.66±17.01	105.33±06.66	16.33±04.51
Zebra fish	45.00±03.00	63.66±14.84	13.33±02.08
Rosy barb	50.66±08.33	84.33±11.68	28.33±04.72
Molly	48.00±03.60	42.33±15.69	17.66±04.16

Table 5: Analysis of fish species' consumption rates per hour for various feeds (95% confidence interval)

Source	Type III Sum of Squares	DF	Mean Square	F value	P value
Corrected Model	41701.111	14	2978.651	50.127	P<0.05
Intercept	67667.222	1	67667.222	1138.753	P<0.05
Fish	14535.556	4	3633.889	61.154	P<0.05
Feed type	20170.711	2	10085.356	169.724	P<0.05
Fish * Feed type	6994.844	8	874.356	14.714	P<0.05

Table 6: Analysis of fish species' consumption rates for various feeds over a three-hour period (95% confidence interval)

Source	Type III Sum of Squares	DF	Mean Square	F value	P value
Corrected Model	75631.867	14	5402.276	56.959	P<0.05
Intercept	150337.8	1	150337.8	1585.099	P<0.05
Fish	20372.533	4	5093.133	53.7	P<0.05
Feed type	40409.2	2	20204.6	213.029	P<0.05
Fish * Feed type	14850.133	8	1856.267	19.572	P<0.05

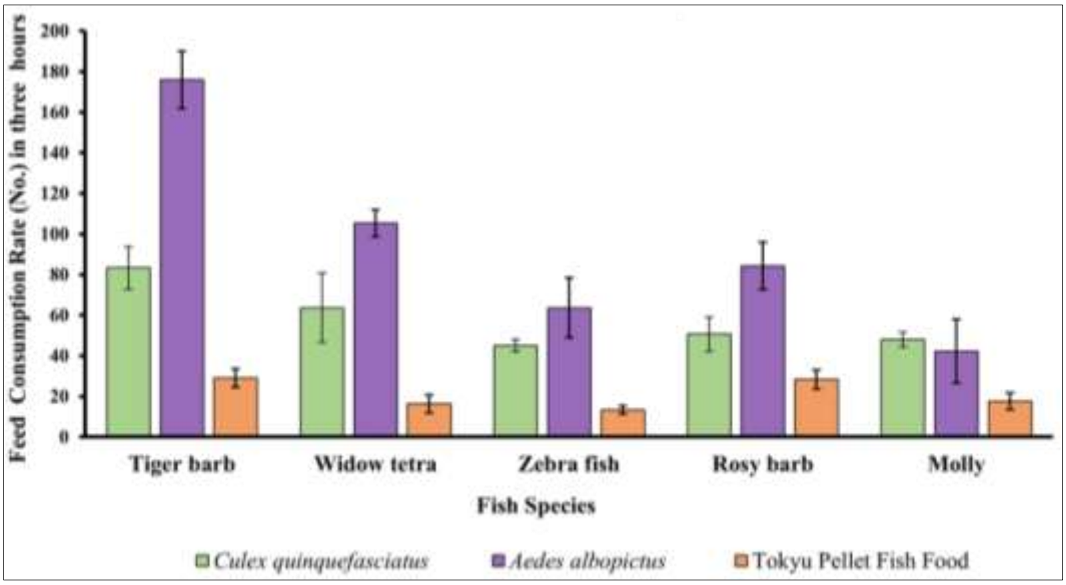

Fig 6: Consumption rate (number in three hours) for three different feeds among five fish species. (Data represented here is Mean±SD)

3.2 Discussion

The findings of the present study provide clear evidence that ornamental fishes generally exhibit a preference for live feed over dry fish pellets. This observation aligns with previous research indicating that live prey is more appealing to ornamental fish species [11].

In the study of the larvivorous potential of various fishes, we observed distinct patterns of larval consumption. Tiger barb, Widow tetra, and Zebra fish takes lesser amount of time for consuming *Culex* larvae compared to *Aedes*. This difference in predation efficiency could be attributed to the dark coloration of *Culex* larvae, which is more conspicuous in the aquatic environment, making them easier targets for these fish species in short period of time.

Surprisingly, our results also revealed a contradictory outcome when estimating the larval feeding rate. We observed that *Aedes* larvae were consumed in larger quantities over longer periods of time, possibly because they are smaller in size compared to *Culex* larvae and tend to aggregate, making them easier to catch. Furthermore, the feeding rate of each fish species declines over time on an hourly basis, primarily due to a notably higher feeding rate observed during the initial hour.

Prior research has shown that the introduction of various non-native ornamental fish species into natural habitats has significant adverse ecological consequences, mainly attributed to their invasive nature [9]. In this study, Tiger barb and Widow tetra exhibited superior larval feeding efficiency compared to the other fish species, may be potentially attributed to their slightly larger size. However, since they are non-native to this study area, caution should be exercised before considering their introduction into the wild, as further experiments are necessary to assess their potential impacts. Nevertheless, for non-native ornamental fish species maintained in aquariums, these types of live feeds can be provided to enhance their care and well-being. This type of research has been previously conducted across various study domains, and the outcomes exhibited a degree of resemblance to our current study [12, 15,11, 16].

The Zebrafish and Rosy Barb used in this study, native to South Asia [10], demonstrated a moderate larvivorous capacity against the targeted mosquito vectors. In certain locations, indigenous fish species have demonstrated superior effectiveness compared to introduce counterparts, such as in the case of Australia [13], India [3], Mexico [2] and Iran [14]. Additional investigations concerning their survival capabilities in diverse mosquito habitats could provide valuable insights, potentially leading to the endorsement of these fish species for controlling mosquito larval populations.

4. Conclusion

Mosquito-borne diseases pose a substantial burden to humanity, and the continuous use of insecticides has contributed to the development of resistance, leading to severe outbreaks. Consequently, exploring alternative biological approaches for mosquito control has become imperative. Numerous studies have demonstrated that live feeds are more crucial in promoting growth than commercially available fish feeds. Therefore, offering high-quality food options to ornamental fishes becomes essential, as doing so will effectively aid in controlling the larval mosquito population. Ornamental fishes have been previously introduced into natural ecosystems to control mosquito populations. Since some non-endemic fish exhibit greater larvivorous activity than the endemic species, caution must be exercised when utilizing these non-endemic species to prevent potential invasiveness. Instead, it would be beneficial to focus on researching endemic ornamental fishes and their larvivorous activity against various mosquito species. To effectively utilize ornamental fishes as mosquitovorous agents, it is essential to thoroughly contemplate various aspects, including selecting appropriate species, assessing habitat suitability, and responsible introduction methods. These measures are crucial to avoid any potential adverse effects on local biodiversity. Implementing such findings in the field can effectively aid in controlling these dangerous vectors.

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6. References

1. Wu N, Wang SS, Han GX, Xu RM, Tang GK, Qian C. Control of *Aedes aegypti* larvae in household water containers by Chinese cat fish. Bulletin of the World Health Organization. 1987;65(4):503-506.
2. Martinez-Ibarra JA, Guillén YG, Arredondo-Jimenez JI, Rodriguez-Lopez MH. Indigenous fish species for the control of *Aedes aegypti* in water storage tanks in Southern Mexico. Bio Control. 2002;47:481-6.
3. Rao JC, Rao KG, Raju CS, Simhachalam G. Larvicidal efficacy of four indigenous ornamental fish species of lake Kolleru, India. Journal of Biodiversity and Environmental Sciences. 2015;7(1):164-172.
4. World Health Organization. Regional Office for the Eastern Mediterranean. Use of fish for mosquito control; c2003. https://apps.who.int/iris/handle/10665/116355. 22 March, 2023.
5. Job TJ. An investigation on the nutrition of the perches of the Madras coast. Records of the Zoological Survey of India, Madras. 1940;42:289-364.
6. Update of annotated bibliography of papers relating to the control of mosquitos by the use of fish for the years 1965-1981/by John B. Gerberich. World Health Organization. https://apps.who.int/iris/handle/10665/62431. 14 April, 2023.
7. Tilak R, Dutta J, Gupta DK. Prospects for the use of ornamental fishes for mosquito control: A laboratory investigation. Indian Journal of public health. 2007;51(1):54-55.
8. Datta A, Sen R, Banerjee R, Banerjee PK. Larvicidal efficacy of ornamental fishes to control *Aedes aegypti* in West Bengal. International Journal of Mosquito Research. 2022;9(4):39-46.
9. Chandra G, Bhattacharjee I, Chatterjee SN, Ghosh A. Mosquito control by larvivorous fish. Indian Journal of Medical Research. 2008;127(1):13-27.

10. Alderton D. Encyclopedia of aquarium and pond fish. Dorling Kindersley Ltd., 2019.
11. Sen R, Datta A, Banerjee R, Sanyal S, Banerjee PK. A comparative study of consumption of *Aedes aegypti* and *Aedes albopictus* larvae by ornamental fishes to control their population in West Bengal. Journal of Entomology and Zoology Studies. 2022;10(5):293-300.
12. Miraldo MC, Pecora IL. Efficiency of brazilian native ornamental fishes as mosquito larvae predators. Boletim do Instituto de Pesca. 2017;44:93-98.
13. Russell BM, Wang J, Williams Y, Hearnden MN, Kay BH. Laboratory evaluation of two native fishes from tropical North Queensland as biological control agents of subterranean *Aedes aegypti*. Journal of the American Mosquito Control Association-Mosquito News. 2001;17(2):124-126.
14. Shahi M, Kamrani E, Salehi M, Habibi R, Hanafi-Bojd AA. Native larvivorous fish in an endemic malarious area of southern Iran, a biological alternative factor for chemical larvicides in malaria control program. Iranian Journal of public health. 2015;44(11):1544-1549.
15. 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(3):270-274.
16. Banerjee R, Datta A, Sen R, Kumar P. Role of ornamental fishes to control *Aedes albopictus* larvae population. 2022;9(6):144-148.
17. QGIS.org, %Y. QGIS Geographic Information System. QGIS Association. http://www.qgis.org. 21 April, 2023.