IJTID. Volume 12 No 3 September-December 2024
Sinta 2 (Decree No: 105/E/KPT/2022)
e-ISSN: 2356-0991; p-ISSN:2085-1103
Available online at https://e-journal.unair.ac.id/IJTID

Original Article

IJTID

(INDONESIAN JOURNAL OF TROPICAL AND INFECTIOUS DISEASE)

Scientific Journal of Tropical and Infectious Disease

Predation Time for Halfmoon and Multicolor Plakat of Varieties of Betta Fish Against *Aedes aegypti* Larvae in Different Water Volume

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OPEN ACCESS

ARTICLE INFO

Received: April 2, 2024
Accepted: October 25, 2024
Published: December 30, 2024
Available online: December 30, 2024

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Keywords:
Aedes aegypti
Betta fish
Halfmoon
Multicolor Plakat
Water Volume

Abstract

One biological strategy for controlling mosquito vectors is using larvivorous fish as larvae predators. Larvivorous fish are an alternative to overcome the problem of larval resistance to temephos. Among the many varieties of Betta fish, the specific predation rates associated with each variety and their behavior in different water volumes remain unclear. This study aims to analyze the differences in predation time for halfmoon and a multicolor plakatof varieties of Betta fish against *Aedes aegypti* larvae in different water volumes.. The research was conducted as a laboratory experiment using a post-test-only design with five replications. Four treatment groups were established, each consisting of one aquarium filled with a specific water volume, one fish, and 25 *Ae. aegypti* larvae. The tests began at 12:00 WIB, and the predation time was recorded until all larvae were consumed. The findings showed that all All varieties of Betta fish can typically predate *Ae. aegypti* larvae. Halfmoon and multicolor plakat have the same predation ability against *Ae. aegypti* larvae ($p > 0.05$). The Mann Whitney's results indicated that Betta fish placed in water with a water volume of 1 and 3 liters had no significantly different predation against *Ae. aegypti* larvae ($p > 0.05$). However, the Kruskall-Wallis test results showed a significant difference in predation abilities between the two varieties when exposed to different water volumes ($p < 0.05$) . The multicolor plakat variety displayed the fastest predation time in 1 liter of water, whereas the halfmoon variety predated more quickly in 3 liters of water.

Cite this as: Adrianto, H., Ritunga, I., Silitonga, H.T.H., Ibrahim, S.N.M.M. (2024). Predation Time for Halfmoon and Multicolor Plakat of Varieties of Betta Fish Against *Aedes aegypti* Larvae in Different Water Volume. *Indonesian Journal of Tropical and Infectious Disease*, 12(3):180–189. https://doi.org/10.20473/ijtid.v12i3.56498

INTRODUCTION

The *Aedes aegypti* mosquito significantly impacts human health by disrupting daily life, feeding on human blood, and serving as a vector for various diseases, including dengue, chikungunya, yellow fever, filariasis, Zika, and West Nile. Dengue hemorrhagic fever (DHF) is among the most severe public health challenges in Indonesia, caused by the dengue virus, which has four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4.[1-4] *Aedes aegypti* spreads the virus from person to person, with the first dengue fever cases in Indonesia reported in Jakarta and Surabaya in 1968. Since then, dengue has been found in all provinces, with infection rates increasing during the rainy season. DHF continues to cause outbreaks and fatalities across numerous districts and cities, with cases rising annually.[5-6]

Currently, there are no human dengue vaccines or antiviral treatments available.[7] Without effective therapeutic drugs, the best approach to preventing and managing the transmission of Aedes-borne arboviruses involves eliminating mosquito habitats and targeting larvae and adult mosquitoes through physical, biological, and chemical control methods.[4,8,9] Chemical insecticides, such as temephos, have been widely used for *Aedes aegypti* management in Indonesia since 1980. Temephos is commonly applied to water reservoirs, which are critical habitats for *Aedes aegypti* larvae.[10,11]

However, prolonged use of temephos has several drawbacks, including the development of resistance in *Aedes aegypti* larvae and environmental pollution due to its persistence.[10,12,13] Resistance to temephos and other insecticides has been reported in Indonesia and several other countries, including Singapore, Malaysia, Thailand, India, and Peru.[14] Despite these challenges, comprehensive data on insecticide resistance across all Indonesian provinces remains limited.[15] To address resistance, strategies such as insecticide rotation and alternative biological vector management should be considered.[16]

The Ministry of Health Indonesia has implemented several strategies to reduce dengue hemorrhagic fever (DHF), including the PSN 3M Plus program (draining, covering, burying, or recycling), using mosquito repellents, keeping larvivorous fish, applying larvicide, and promoting the G1R1J movement (1 House 1 Jumantik).[17-19] Among these, keeping larvivorous fish is emphasized as an effective and user-friendly biological control method.17 Several species, including *Gambusia affinis*, *Poecilia reticulata*, and *Betta splendens*, have been identified as potential predators of *Aedes aegypti* larvae.[20-22] Notably, Betta splendens thrives in low to moderate temperatures, making it well-suited for various regions in Indonesia.[22]

Betta splendens, commonly known as Siamese fighting fish or "betta," is a freshwater species renowned for its physical diversity and aggressive behavior.[23] Over 600 years of domestication have led to variations in pigmentation, body size, and fin shapes, with popular strains including halfmoon, crown tail, double tail, and plakat.[24,25] The diverse color patterns and tail shapes of B. splendens can lead to species selection errors, and thus far, predation studies have focused on *B. splendens* as a species without distinguishing between varieties.[28-30] Specific information on which varieties are more effective at preying on mosquito larvae remains unclear and needs further exploration.

While *B. splendens* is recognized for its larvivorous potential, research on the differences in predation efficiency among its varieties is limited. Our previous study identified the crown tail (Serit) variety as a promising predator, with an average feeding time of 3 minutes and 30 seconds to consume mosquito larvae, and a maximum feeding duration of 4 minutes.[31] In this study, we aim to investigate the predation efficiency of two other betta varieties—halfmoon and multicolor plakat—against *Aedes aegypti* larvae in different water volumes to determine their potential as effective biological control agents.

MATERIALS AND METHODS

Samples Preparation

Male halfmoon and multicolor plakat variants of *B. splendens*, measuring 3.5-5.5 cm from mouth to tail fin, were palced in an aquarium and fed twice daily. Acclimatization lasted for one week to allow the fish to adjust and avoid stress in their new surroundings. Fish were fasted for one day before testing to ensure their predation ability was not compromised by previous satiety. The Service Unit Identification at the Faculty of Marine and Fisheries, Universitas Airlangga, uses the identification certificate 56/ULMKILP/ UA.FPK/09/2023 to identify the fish. Third-instar *Ae. aegypti* larvae were procured from the Entomology Laboratory, Health Polytechnic, Ministry of Health Surabaya.

Fish Predation Testing

This study used four treatment groups. The first group contains the halfmoon variety in an aquarium with a water volume of 1 liter. The second group of halfmoon varieties in an aquarium with a water volume of 3 liters. The third group of multicolor plakat varieties in an aquarium with a water volume of 1 liter. The fourth group of multicolor plakat varieties in an aquarium with a water volume of 3 liters. Each group had five replications and a total of 20 aquariums. The glass aquarium measures 14 cm long, 14 cm wide, and 24 cm high. Each aquarium contained 25 individual *Ae. aegypti* larvae. The research was carried out during the day (12.00 WIB).

When the larvae reached the water, the timer was activated. The time taken for the *Betta* fish to predate all of the *Ae. aegypti* larvae was recorded.

Data Analysis

The data were examined using SPSS™ 25 statistical software. Data analysis was performed using Mann-Whitney and Kruskal-Wallis tests, with post-hoc follow-up tests being included. Statistically significant differences were determined when the p-value was less than 0.05.

RESULTS AND DISCUSSION

All betta fish in this study can generally predate all *Ae. aegypti* larvae. The Shapiro-Wilk normality test revealed that the predation data for multicolor plakat had a normal distribution with a p-value of 0.129 ($p > 0.05$). However, the halfmoon data was not normally distributed with a p-value of 0.027 ($p < 0.05$). The Levene test revealed that the two varieties of Betta fish had a homogenous variance in predation time, with a p-value of 0.225 ($p > 0.05$). The results of the test for differences in halfmoon and multicolor plakat predation times on *Ae. aegypti* larvae were analyzed using the Mann-Whitney test (Table 1).

Tabel 1. Mann-Whitney Analysis Result

Variety	Mean	SD	*p*
Halfmoon	4,85	2,40	0,519
Multicolor plakat	4,85	3,51	

Mann-Whitney (* = Significantly different at 5% significance level)
SD = Standard Deviation

Table 1 shows that the halfmoon has an average predation againts *Ae. aegypti* larvae of 4.85 minutes with a median of 3.75 minutes. On average, multicolor plakat predation time is 4.85 minutes, with a median of 4.25 minutes. Descriptively, it shows that the variety of *Betta* fish with the fastest predation time against *Ae. aegypti* larvae is the halfmoon because it has a lower median, even though the average predation time is the same as the multicolor plakat. The Mann-Whitney test showed that the two types of fish studied had no significantly different predation abilities in preying on *Ae. aegypti* larvae, as indicated by a p-value 0.519 ($p > 0.05$). Halfmoon (Figure 1) and multicolor plakat (Figure 2) have the same predation ability against *Ae. aegypti* larvae.

Figure 1. Halfmoon variety

Figure 2. Multicolor plakat variety

Results of the Shapiro Wilk normality test during predation of *Betta* fish in a 1 Liter aquarium ($p = 0.012$) and a 3 Liters aquarium ($p = 0.092$). The results of the normality test showed that *Betta* fish placed in a 3 Liters aquarium had normal distribution ($p > 0.05$), while *Betta* fish placed in a 1 Liter aquarium had data that was not normally distributed ($p < 0.05$). The results of the homogeneity test using the Levene test ($p = 0.592$) showed that both aquarium volumes had homogeneous predation time variances ($p > 0.05$). The test for differences in predation time for *Betta* fish on *Aedes* larvae based on aquarium volumes of 1 liter and 3 liters was analyzed using the Mann-Whitney test (Table 2).

Table 2. Mann-Whitney Analysis Result

Volume	Median	Mean	SD	*p*
1 Liter	2,75	4,05	2,95	0,111
3 Liters	5,00	5,65	2,83	

Mann-Whitney (* = Significantly different at 5% significance level)
SD = Standard Deviation

Table 2 shows that *Betta* fish placed in a 1 liter water had an average predation time against *Ae. aegypti* larvae of 4.05 minutes with a median of 2.75 minutes. On average, *Betta* fish placed in a 3 liters water have a predation time of 5.65 minutes with a median of 5 minutes. Descriptively, it shows that the *Betta* fish had the fastest predation time against *Ae. aegypti* larvae are those placed in 1 liter water because they have a lower mean and median.

Mann Whitney's results showed that the two volumes of water studied had no significantly different predation abilities against *Ae. aegypti* larvae, as indicated by a value of p = 0.111 (p > 0.05). These results mean that *Betta* fish placed in water with a volume of 1 and 3 liters have the same predation ability in preying on *Ae. aegypti* larvae.

The difference in predation time for halfmoon and multicolor plakat placed in 1 liter and 3 liters water against *Aedes* larvae was analyzed using the Kruskall Wallis test and followed by the Mann-Whitney test. Shapiro Wilk normality test results during predation of halfmoon in a 1 liter water (p = 0.311), halfmoon in a 3 liters water (p = 0.421), multicolor plakat in a 1 liter aquarium (p = 0.146), and multicolor plakat in a 3 liters aquarium (p = 0.216). The normality test results showed that all groups of varieties of *Betta* fish had a normal distribution (p > 0.05). The results of the homogeneity test using the Levene test (p = 0.003) showed that the predation time data for the two varieties in 1 liter and 3 liters water had a variance in predation time that was not homogeneous (p < 0.05). The results of the test for differences in predation time based on the types of halfmoon and multicolor plakat in 1 liter and 3 liters water against *Ae. aegypti* larvae were analyzed using the Kruskall Wallis test.

Multicolor plakat in 1 liter water has the fastest predation time against *Ae. aegypti* larvae. Halfmoon has a faster predation time when placed in 3 liters of water. The results of the Kruskall Wallis analysis showed that the two groups of fish with different water volumes had significantly different predation abilities against *Ae. aegypti* larvae, as indicated by the p-value = 0.005 (p < 0.05) (Table 3).

Table 3. *Kruskall Wallis* Analysis Result

Variety	Volume water (L)	Mean	*p*
Halfmoon	1	6,10	0,005*
Halfmoon	3	3,60	
Multicolor plakat	1	2,00	
Multicolor plakat	3	7,00	

Mann-Whitney (* = Significantly different at 5% significance level)

Table 4 shows that further tests using the Mann-Whitney test to find out which group had the fastest predation time compared to other groups showed that halfmoon in a 1 liter water volume and halfmoon in a 3 liters water volume had predation abilities that were not significantly different (p>0.05). Halfmoon in 1 liter water had a significantly different and longer predation time than the multicolor plakat in 1 liter water (p < 0.05). Halfmoon in 3 liters water had a long predation time that was significantly different from multicolor plakat in 1 liter water (p = 0.014) and multicolor plakat in 3 liters water (p = 0.009) but was faster than multicolor plakat in 3 liters water (p < 0.05). Multicolor plakat in 1 liter water had a significantly different predation time than in 3 liters water (p = 0.009). Multicolor plakat in 1 liter water had a faster predation time than multicolor plakat in 3 liters water (p < 0.05).

Table 4 Mann Whitney Test Results of Predation Time of Halfmoon and Multicolor Plakat Fish in 1 liter and 3 liters Volumes Against *Ae. aegypti* Larvae

Group	A	B	C	D
A	-	-	-	-
B	0,293	-	-	-
C	**0,016***	**0,014***	-	-
D	0,690	**0,009***	**0,009***	-

* = Significantly different at 5% significance level)
A = Halfmoon in 1 liter water
B = Halfmoon in 3 liters water
C = Multicolor plakat in 1 liter water
D = Multicolor plakat in 3 liters water

Overall, the combination of *Betta* fish varieties and water volume had a significant effect the predation time of *Ae. aegypti* larvae. The *Betta* fish varieties have the best predation time against *Ae. aegypti* larvae are multicolor plakat, placed in 1 liter water, followed by halfmoon in 3 liters of water. Lastly, multicolor plakat in 3 liters of water and halfmoon in 1 liter water have the same capabilities.

The variety capabilities of *Betta* fish in this study are like previous studies. The Serit variety (crown tail *Betta*) of *Betta* fish has potential as a larvivorous fish because it can predate all 25 individual third instar *Ae. aegypti* larvae each replication.[31] The results of different larval predation abilities based on varieties are also like the results of previous studies. The multicolor plakat variant of *Betta fish* suggested a faster predation time compared to the single-colored plakat variant of *Betta fish*. A single-colored plakat variant can consume 25 *Ae. aegypti* larvae in an average time of 3.4 minutes. The multicolor plakat variant can consume 25 *Ae. aegypti* larvae in an average of 2.7 minutes.[32] Male and female fish consumed more mosquito larvae during the daytime than at night. The availability of light influenced the predator's eating rate since it made it easier to seek for and attack the prey. The water volume, prey species, number of fish predators available, prey densities, and sex of the prey all had an impact on predation activities.[33] Water volume also has an impact on predation and feeding rates. When 2 liters of water were utilized in a previous study, predation and feeding rates fell. The fish spent more time feeding and looking for mosquito larvae. The feeding rate was reduced as the volume of water increased, but it increased when the number of predators and prey densities increased.[33] With the increase in the volume of water, the predation rate lowered, but the prey consumption increased linearly with the prey density irrespective of water volume. The consumption of mosquito larvae at a particular prey density is reduced with an increased volume of water, possibly due to the evasion tactics of the mosquitoes.[34]

This study predicted that the size of the tail fin influences the swimming speed and prey of larvae. Body size and tail type affected swimming speed.[35] Fish's capacity to swim is influenced by the morphology of their tail fins, body shape, and habitat. The tail beat is a significant factor in fish swimming speed. Furthermore, it determines fish swimming endurance, which can be used to distinguish between maximum sustained, protracted swimming speed and maximum (burst) swimming speed. The fish's ability to swim is mainly determined by its tail fin. The movement of a fish's caudal fin relates to energy intake and body metabolism.[36]

Betta splendens fish are the most effective predators at medium and lower temperatures. They have adapted to lower temperature levels. Due to temperature variations ranging from standard to low, this species' predatory efficiency remains reasonably steady.[22] Male *Betta* fish were chosen in this study because male fish have more beautiful bodies, so they are more popular than female *Betta* fish, they are sold and kept more often.[37]

Previous research reported that an average predation ability of 85.87% discovered a significant difference in the quantity of *Ae. aegypti* larvae while bathing before and after adding *Betta* fish. The acceptance rate and sustainability of utilizing *Betta* fish as a biocontrol agent achieved 96.67%, and the community has a very favorable opinion of their use. Taste, color, and scent did not alter the properties of the water.[30]

Larvivorous fishes are not only implemented in bathtubs or water reservoirs in the house. Larvivorous fishes can thrive in both artificial and natural environments, including water tanks, lakes, fountains, pools, cattle troughs, swimming pools, water storage tanks, seepage, irrigation cisterns, canals, shallow pools, small dams, rice fields, ponds, riverbed pools, slow-moving streams, swamps, and temporary water collection systems.[38]

The use of larvivorous fish is a simple approach of eliminating vector mosquitos.[22] Effectiveness of *Betta* Fish *Betta splendens*, as a biological predator of *Ae. aegypti* larvae, can be utilized to control *Ae. aegypti* larvae in a safer, faster, more cost-effective, and community-wide manner. The efficient use of this fish is likely to reduce the amount of Dengue Hemorrhagic Fever patient occurrences.[29] Aside from that, larvivorous fishes can be helpful to on a vast scale that is commercially viable and pollution-free.[20]

STRENGTH AND LIMITATION

The advantage of this research is that the ability of *Betta* fish against *Ae. aegypti* larvae are more apparent based on variety. We can determine which varieties are more appropriate to implement in the 3M Plus program. A limitation of this study is that no fish other than *Betta* were compared as a control group. The varieties studied still use two varieties, so further research is needed on other varieties of *Betta* fish in the future.

CONCLUSIONS

The combination of *Betta* fish varieties and water volume had a significant effect on the predation time of *Ae. aegypti* larvae.

ACKNOWLEDGEMENT

The authors would like to acknowledge the School of Medicine, Universitas Ciputra, Surabaya, Indonesia, for providing us with the resources and support needed to complete this project. We also thank Rytney Electra Sheyoputri, Gianina Angelia Santoso, Faza Budargo, Amadeus Michel Goein, Jonathan Loody Lukas, Martha Vinda Candra Juwono, Bracovanca Diwayestara Bravimasta, and Ian Ardhiya Firmanto, to support us in carrying out measurements and collecting data in the laboratory. These contributions were invaluable in helping us complete this project and draw meaningful conclusions.

ETHICAL CLEARANCE

The research protocol was approved by the Health Research Ethics Committee of the Hang Tuah Medical Faculty, Surabaya, number I/120/UHT.KEPK.03/IX/2023.

FUNDING

This paper did not receive nor use any sorts of funding.

CONFLICT OF INTEREST

The authors declare that this manuscript was approved by all authors, and that no competing interests exist.

AUTHOR CONTRIBUTION

Conception, design, and/or analysis and interpretation of data, drafting the article: HA. Discussion, and critical revision for important intellectual content: IR and HTHS. Review: HA.

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