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Original Article

Male and Female Rosy Barb Fish (*Pethia conchonius*) Predation Time Against *Aedes aegypti* Mosquito Larva and Pupa Stage in the Morning

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ABSTRACT

Introduction: The control program for Dengue Hemorrhagic Fever (DHF) outbreaks prioritizes controlling the mosquito vector population. One way of biologically controlling mosquitoes that is safer and more environmentally friendly is fish as natural predators of larval and pupae stage mosquitoes. One fish tested in this study was Rosy Barb (*Pethia conchonius*). **Objective:** This study aimed to compare the male and female fish predation ability of rosy barb (*P. conchonius*) in *Aedes aegypti* larval and pupal stages. **Methods:** This study is a laboratory experiment with a post-test-only design and five replications. One aquarium holds one litre of water, one fish, and 25 *Ae. aegypti* larval/pupa tails. Fish testing begins at 09.00 WIB in the morning. Fish predation was timed until the fish consumed all larvae and pupae. **Results:** The mean time of predation of male *P. conchonius* to larvae was 12.03 minutes, and pupae were 2.83 minutes. The mean time of predation of female *P. conchonius* on larvae was 11.70 minutes, and pupae were 4.28 minutes. The results of the independent sample t-test equal variance assumed statistical test showed that male and female fish had mosquito larvae feeding times that were not significantly different ($p > 0.05$). Male and female fish had mosquito pupa feeding times that were not significantly different ($p > 0.05$). **Conclusions:** Rosy barb fish (*P. conchonius*) has the potential as a natural predator of *Ae. aegypti* larval and pupal stages.

Key words: *Aedes aegypti, Pethia conchonius*, Male fish, Female fish, Larvae, Pupae.

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INTRODUCTION

The *Aedes* mosquito is a genus of mosquitoes important in Indonesia. The *Aedes* mosquito is a vector of arthropod-borne diseases for dengue virus, chikungunya, zika, yellow fever viruses, and sometimes filarial worm.[1,2] According to the World Health Organization, dengue fever is the most salient mosquito-borne viral disease and public health importance in tropical and temperate regions of the world.[3] The first dengue outbreak was reported in Surabaya and Jakarta, Indonesia, in 1968.[4] Indonesia has two crucial Aedes mosquitoes: *Aedes aegypti* and *Aedes albopictus*.[5,6]

The Indonesian Ministry of Health report said that dengue fever cases in 2021 were 73,518 cases. The DHF mortality rate in 2022 is 705 deaths.[7] From these problems, it is necessary to control mosquito vectors to reduce the incidence of morbidity and mortality. The control program for Dengue Hemorrhagic Fever (DHF) outbreaks prioritizes controlling the mosquito vector population. Until now, controlling dengue fever has relied on reducing potential breeding sites at home and chemical control of *Aedes aegypti* larvae and adults.[8] Organophosphate temephos is a commonly used larvicide for public health interventions. Chemical control of the *Aedes* mosquito has declined due to insecticide resistance and environmental consequences, such as vector resistance and toxicity to humans and non-target organisms.[9,10] Using insecticides for mosquito control, including organophosphates, carbamates, and pyrethroids, can also adversely affect human health.[11] A previous study by Haziqah-Rashid shows that all *Ae. aegypti* larvae from 10 study sites in Indonesia (Kuningan, Padang, Samarinda, Pontianak, Denpasar, Mataram, Dompu, Manggarai Barat, East Sumba, and South-Central Timor) resistant to diagnostic doses of temephos larvicide with mortality rates ranging from 0 to 76%.[4] Three sub-districts in Surabaya (Tambaksari, Gubeng and Sawahan) reported the mortality rate of *Ae. aegypti* larvae are below 80%, which indicates possible resistance to temephos.[12] in another country, Laos, Cambodia, Thailand, Vietnam, Brazil, Pakistan, and Peru have reported temephos-resistant *Aedes aegypti* larvae.[13-15]

Scientists continue exploring new methods to control mosquito populations from this problem. One of the alternative vector controls to overcome resistance to temephos using biological control.[16] Living things that can be used as biological control are Bti granules, larvivorous fish, *Toxorhynchites* mosquito larvae, and larvivorous copepods.[11] One way of biologically controlling mosquitoes that is safer and more environmentally friendly is fish as natural predators of larval and pupae stage mosquitoes. These larvivorous fish were intentionally introduced to control mosquitos in the United States prior to the introduction of pesticides in 1921, but their use was greatly reduced after the widespread use of effective chemicals. Because of the development of resistance in mosquitos to chemical pesticides, the use of larvivorous fish to control mosquitos is being reconsidered.[17] Many studies from various countries report that larvivorous fish have successfully controlled Anopheles larvae in a variety of habitats around the world.[18] Some

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fish that have been recognized as larvivorous fish and applied in the community are *Gambusia affinis, Poecilia reticulata, Carassius auratus,* and *Aplocheilus lineolatus*.[11] One fish tested in this study was rosy barb (*Pethia conchonius*). Male *P. conchonius* fish has a distinctive characteristic: a coloured body orange and a belly slimmer, while the female fish is paler in colour and has a more rounded belly. This fish has a black colour at the tip of the dorsal fin and tail base. Rosy Barb fish are easy to find at the fish market and shop, with a cheap price of 3,000-4,000 rupiah.[19] Previous studies reported rosy barb male sex has a faster predation ability against third-instar larvae of *Ae. aegypti* than other fish (female rosy barb, male and female lemon fish) in the evening (15.00 WIB).[19]

This study aimed to compare the male and female fish predation ability of rosy barb fish (*P. conchonius*) in *Aedes aegypti* larval and pupal stages in the morning.

MATERIALS AND METHODS

Ethical considerations

This research is an experimental study in a laboratory with a post-test-only design method. The Ethical Committee Medical Research Universitas Ciputra Surabaya, Indonesia, approved this study (No.140/EC/KEPK-FKUC/XII/2021).

Fish and mosquito preparation

Male and female rosy barb fish (*P. conchonius*) can be found at the Geluran-Taman fish shop in Sidoarjo. The fish tested had a length of 5-5.5 cm from the mouth to the tip of the tail fin. Fish determination is carried out in the Service Unit Identification at the University's Faculty of Marine Fisheries Airlangga. The mosquitoes tested were species of *Ae. aegypti* larval stage (third-instar larvae) and pupa stage. Larvae and pupae of *Ae. aegypti* obtained from the Entomology Laboratory, Institute of Disease Tropical, Universitas Airlangga Surabaya.

Fish predation testing

Before the study began, the fish were adjusted (acclimatization) first. This acclimatization begins by placing the fish in the aquarium for one week. Fish were fed fish pellets twice a day. One day before testing, the fish were not fed for one day. On the day of testing, one fish was included in one 14 cm (length) x 14 cm (width) x 24 cm (height) aquarium containing two litres of tap water. Larvae or pupae of *Ae. aegypti*, as much 25 tails are put into an aquarium that already contains fish. After the mosquito larvae or pupae are poured into the aquarium, we record the time the fish eats the larvae or pupae until they run out.

Statistical analysis

Differences in feeding time of male and female fish to mosquito larvae and pupae will be analyzed using parametric statistics, namely independent sample t-test, with the condition that the feeding time data has a normal distribution and homogeneous data variance. The normality test will be analyzed using Shapiro-Wilk, while the homogeneity test will be analyzed using the Levene test. The feeding time difference test will be analyzed using non-parametric statistics (Mann-Whitney test) if the data is not normally distributed. Suppose the data is normally distributed but has inhomogeneous data variance. In that case, the feeding time difference test is analyzed using an independent sample t-test with equal variance not assumed.

RESULTS

Rosy barb fish (P. *conchonius*) predation time to larvae and pupae of Ae. *aegypti*

The mean time of predation of male *P. conchonius* to larvae was 12.03 minutes, and pupae were 2.83 minutes. The mean time of predation of female *P. conchonius* on larvae was 11.70 minutes, and pupae were 4.28 minutes.

Feeding time for male and female fish against mosquito larvae

The results of the Shapiro-Wilk normality test when eating male ($p = 0.176$) and female ($p = 0.050$) fish on mosquito larvae had data that were normally distributed ($p > 0.05$). At the same time, the results of Levene's homogeneity test showed that both had homogeneous data variance ($p = 0.674 > 0.05$). This means that both meet the requirements of using an independent sample t-test. The following are the results of the unpaired data t-test (independent sample t-test).

The average feeding time of male fish against mosquito larvae is 722.20 $\pm$ 874.75 seconds, with the fastest feeding time being 48 seconds and the longest feeding time being 2147 seconds. The female fish had a slightly faster average feeding time of mosquito larvae, namely 702.20 $\pm$ 957.55 seconds, with the fastest feeding time of 52 seconds and the longest feeding time of 2188 seconds. Both have a 20-second mealtime difference. The results of the independent sample t-test equal variance assumed statistical test showed that male and female fish had mosquito larvae feeding times that were not significantly different ($p = 0.973 > 0.05$). This means that male and female fish have the same average feeding time for mosquito larvae [Table 1].

Feeding time for male and female fish against mosquito pupae

The results of the normality test of Shapiro Wilk when eating male ($p = 0.164$) and female ($p = 0.392$) fish against mosquito pupae had data that were normally distributed ($p > 0.05$). Meanwhile, Levene's homogeneity test results showed that both had homogeneous data variances ($p = 0.476 > 0.05$). This means that both meet the requirements of using an independent sample t-test. The following are the results of the unpaired data t-test (independent sample t-test).

Data from Table 2 showed the average feeding time of male fish against mosquito pupae is 169.80 $\pm$ 61.65 seconds, with the fastest feeding time being 112 seconds and the longest feeding time being 274 seconds. The female fish had a slightly longer average feeding time of mosquito pupae which was 257.20 $\pm$ 104.95 seconds, with the fastest feeding time of 147 seconds and the longest feeding time of 429 seconds. Both have a difference in feeding time of 87.4 seconds. The results of the independent sample t-test equal variance assumed statistical test showed that male and female fish had mosquito pupa feeding times that were not significantly different ($p = 0.147 > 0.05$). This means that male and female fish have the same average feeding time of mosquito pupae.

DISCUSSION

This study suspected that fish have a predation rate based on fish sex and time. Previous study reports found that female fish *Aplocheilus*

Table 1: Average feeding time of male and female fish against mosquito larvae (second).

Groups	n	Min	Max	Mean $\pm$ SD	Difference	p
Male fish	5	48	2147	722,20 $\pm$ 874,75	20	0,973
Female fish	5	52	2188	702,20 $\pm$ 957,55		

Independent sample t-test equal variance assumed

Table 2: Mean feeding time of male and female fish against mosquito pupae (second).

Groups	n	Min	Max	Mean $\pm$ SD	Difference	p
Male fish	5	112	274	169,80 $\pm$ 61,65	87,4	0,147
Female fish	5	147	429	257,20 $\pm$ 104,95		

Independent sample t-test equal variance assumed *panchax* eats larvae faster (6 minutes 44 seconds) than male fish.[20] There is a significant difference between male and female *P. conchonius* group predation in the evening. On the other hand, there is no significant difference between the predation of male and female *L. caeruleus* fish groups against third-instar larvae of *Ae. aegypti* in the evening.[19] Fish history has identified mosquito larvae as previous prey, likely influencing the speed of fish predation. Lighting, room temperature, and aquarium size are all potential factors that influence fish predation speed.[21] Another study found that a fish of predator's effectiveness is determined by its weight and sex of fish. Both tilapia species, Wild and GIFT, consume more larvae in the morning than in the evening. The daily appetite rhythm of larvivorous fish follows a pattern, with the first peak in the morning and the second peak in the afternoon/evening.[22] *Rasbora daniconius* and *Colisa fasciata* consumed more larvae during the day than at night in all water volumes, according to another study. In contrast to *Pseudomugil signifer*, the only species that did not show a significant reduction in larval consumption during the night experiments.[23] The predation power of fish can also be affected by several factors that are difficult to control, such as survival in harsh environmental conditions.[24]

The ability of larvivorous fish to eat up mosquito larvae and pupae will reduce the potential for the mosquito life cycle to reach the adult stage (imago) and transmit the virus to healthy people. The ability of rosy barb fish in this study needs to be implemented in the community. Ghosh said that larval fish in natural habitats that feed on *Anopheles* larvae had been used successfully in malaria control.[25] Giving betta fish proved to be effective in reducing the number of larvae in Talok Village, Turen District.[26] The using of larvavirous fish is not hazardous to plants, beneficial insects, or human health.[22] In this study, the *P. conchonius* fish predation time was faster when preying on the pupal stage of *Aedes aegypti* than the larvae. Previous research found that the small and large larvae of *Cx. pipiens* consumed daily by *O. niloticus* were significantly taller than the pupae.[27] As part of the vector management strategy in the endemic region, periodic surveys and monitoring of fish biodiversity, demarcation of breeding sites, field level research study on the efficacy of larvavirous fishes, and public awareness on the establishment of larvivorous fishponds should be implemented.[28]

Potential larvivorous Poecilidae, Cyprinidae, Cyprinodontidae, and Chichlidae are known and used. In 1905, *Gambusia* affinis was introduced from Texas to Hawaii. In the 1920s, Spain and Italy. In the 1920s, it expanded to 60 countries. In 1908, *Poecilia reticulata* (Guppy) was introduced from South America to British India.[29] Guppies have the potential to be an effective natural biological vector control for *Aedes aegypti, Aedes albopictus*, and *Culex quinquefasciatus*.[30] For a fish to be used as a mosquito biocontrol agent, it must meet two basic criteria: First, it must have a high predation efficacy on mosquito larvae; second, it must be resistant to low oxygen concentrations while also being toxic metabolite tolerant.[31] In future research, it is expected that the potential of rosy barb fish needs to be tested on other species of mosquito larvae. Larvivorous fish for *Aedes aegypti* in the field are still rarely found.[32] Larvivorous fish data needs to be dug more profound so that scientific information on predatory fish data can be obtained and help the government tackle dengue disease. Larvivorous fishes can play an important role in mosquito larvae control.[33] The effectiveness, pollution free, economically viable, and low cost of incorporating fish rearing into community-based health structures suggest that they should be considered as a vector control tool as long as the benefits outweigh any potential environmental concerns.[34,35]

The limitations of this study are that we have not tested how many larvae and pupae can be consumed by fish until they are full, nor the predation patterns of fish in communities with larger water bodies.

CONCLUSIONS

Male and female fish have the same average feeding time ($p > 0.05$) for larvae and pupae mosquitoes. Rosy barb fish (*P. conchonius*) has the potential as a natural predator of *Ae. aegypti* larval and pupal stages. The research results will help the government use rosy barb fish that are more targeted and effectively implemented in society.

SUMMARY

This study reports the rosy barb fish (*P. conchonius*) has the potential as a natural predator of *Ae. aegypti* larval and pupal stages.

CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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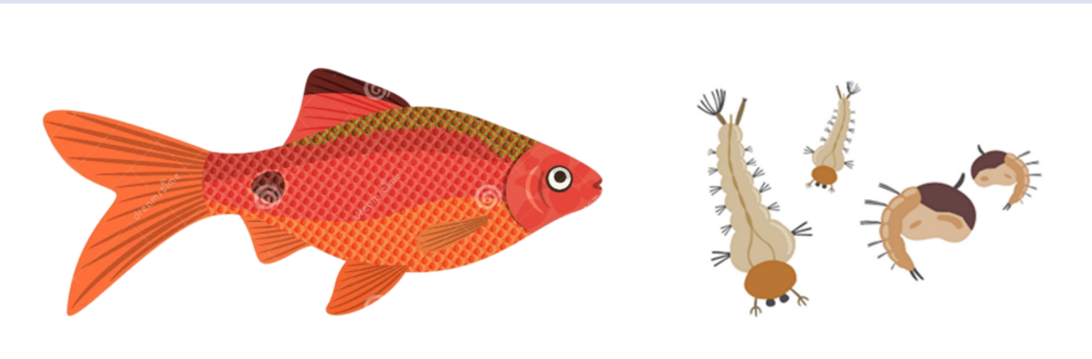
GRAPHICAL ABSTRACT



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