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Original Article

Predatory Efficiency of Larvivorous Fish against Mosquito Larvae in Different Water Temperature Levels: Implication in Control Measure of Dengue Vector

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Abstract

Background: Reduction of the *Aedes aegypti* population is the priority effort to control dengue virus transmission including the use of larvivorous fish. Biologically, the predatory efficiency of fish will slow down when the water acidity and temperature change from normal conditions. This study aimed to determine the predatory efficiency of three species of larvivorous fish against the *Ae. aegypti* larvae in different water temperatures.

Methods: Three well-known species of larvivorous fish namely *Poecilia reticulata*, *Betta splendens,* and *Aplocheilus panchax* were placed into 12 cm diameter jars with three water temperature ranges namely 20–21 ºC, 27–28 ºC, and 34–35 ºC, and allowed to three days acclimatization. As many as one hundred 4th-instars larvae of *Ae. aegypti* were gradually entered into each jar, and a longitudinal observation was made at 5, 10, 30, 60, 120, 240, 360, 480, 600, and 720 minutes. The predated larvae were recorded.

Results: In normal temperature ranges, the predatory efficiency of the larvivorous fish was 75%, 72.3%, and 32.8% for *B. splendens. Aplocheilus panchax*, and *P. reticulata*, respectively. The predation abilities decreased due to temperature changes. *Betta splendens* and *A. panchax* indicated the best predatory efficiency against *Ae. aegypti* larvae in different temperature conditions.

Conclusion: *Betta splendens* is the best larvivorous fish in the lower to normal, but *A. panchax* is the best in the normal to higher temperature ranges. This finding should be considered by public health workers in selecting larvivorous fish to control the Dengue vectors.

Keywords: Predatory efficiency; Larvivorous fish; *Aedes aegypti* larvae; Water temperature

Introduction

Controlling the *Aedes aegypti* larvae is one of the mainstay efforts in reducing the rate of Dengue virus infection in the tropical and subtropics countries that face the problem of Dengue (1) because there is currently no antiviral drug and vaccine availability is still limited (2). At present, around 3.8 billion people spread across 128 countries are at risk of being infected with the dengue virus (3). The affected area can be increasingly expanded due to global warming which causes an expansion of the area that is conducive to the habitat of this vector species (4). The research findings show that the distribution of dengue vectors covers a very wide region including various altitudes from coastal areas to mountains with no different densities (5, 6). This phenomenon is in line with the increase in the daily air temperature which causes an increase in the carrying capacity of the environment to the *Aedes* mosquito viabil-

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ity. In addition to the temperature aspect, the altitude of the place was also related to the availability and adequacy of clean water due to lower rainfall in the year (5). The fulfillment of clean water needs is strived by building large and open water reservoirs (5, 6). This condition caused the hilly and lack of clean water areas to have high support for the Dengue vector viability due to the availability of suitable habitat. The existence of new habitats in the form of the opened-large water reservoirs also caused the complexity of the eradication program of *Ae. aegypti* larvae, in addition to resistance to temephos (7). The large volume of water container needs a high quantity of temephos to control the mosquito larvae. This condition caused the larvae control program to become inefficient. Based on this phenomenon, many studies recommend the use of larvivorous fish as a proper dengue vector eradication program in these areas (5).

Several studies reported that some species of larvivorous fish are effective in eradicating *Ae. aegypti* larvae, such as *Poecilia reticulata* (Peters, 1859), *Betta splendens* (Regan, 1910), and *Aplocheilus panchax* (Hamilton, 1822) (8). So far, the effectiveness of these fish species has been tested with uniform habitat condition and there were no water and temperature variations. In the field, there are wide variation ranges of environmental conditions. There are clear inverse comparisons between the altitudes of the places with the air temperature where the coastal areas are hotter than the mountains with a wide temperature range, almost 10 degrees (5). An increase in the average air temperature increased the water temperature.

In addition to biological conditions, the predatory efficiency of larvivorous fish is affected by habitat environmental conditions including temperature and acidity (pH). The optimum temperature for larvivorous fish varies, namely 24–28 ºC for *P. reticulata* and *B. splendens*, and 24–30 ºC for *A. panchax*, and with a pH range between 6–7 (9–12). The ability of larvivorous fish will slow down at the temperature and pH ranges that are not suitable for the optimum conditions due to physiological disorders. Fish adapt to changes in habitat temperature by more than 5 ºC degrees by inactivity (13). This phenomenon needs to be investigated and the suitability of larvivorous fish species with the environmental conditions must be understood before intervention. This in-vitro study aimed to determine the interaction effect of water temperature and larvivorous fish species on the predatory efficiency against *Ae. aegypti* larvae, as a basis for public health interventions in eradicating Dengue vectors.

Materials and Methods

Larvivorous fish

We used three local species of larvivorous fish that have been understood to have good predatory efficiency namely *P. reticulata*, *B. splendens*, and *A. panchax* in both sexes. The fish were obtained from a fish seller in the traditional market in Semarang City. The fish were selected based on body length with the ranges of 3.5–4.0 cm and actively moving.

Aedes aegypti larvae

We used the 4th instar larvae of laboratory colony *Ae. aegypti* of local strains obtained from settlements in Sendangmulyo village, Semarang City, Central Java Province, Indonesia. As many as 1,200 and 3,600 healthy and actively moving larvae were subjected to the predatory efficiency test in the preliminary study and predatory efficiency (final) experiment.

Experiment conditions

We conducted an experimental study with the factorial design based on the interaction of three species of larvivorous fish and three levels of water temperature. Each experiment group was three times replicated. This experiment implemented three categories of water temperature representing the low, normal/optimum, and high levels, namely at 20–21 ºC, 27–28 ºC, and 34–35 ºC, respectively. The temperature ranges were maintained based on the optimum temperature for mosquito larvae and larvivorous fish, and pH was tested using litmus paper and recorded. Low-temperature groups were obtained by outflanking the ice gel around the jar. Medium/optimum temperature groups were used at the open room temperature, and the temperature of the high level was obtained by emerging the jar into heated water (by the electric water heater). Larvivorous fish habitats were used in the 12 cm diameter of 36 plastic jars. One liter of fresh water was filled into each jar, and one larvivorous fish was placed in each jar one hour later. All the larvivorous fish were acclimated in the jar for three days and fed with fish food concentrate. Food feeding was stopped in the last 12 hours and the extra foods were sucked up clearly from the bottom of the jar by a pipette.

Predatory efficiency

One hundred of the healthy and active movement of the 4th instar larvae of *Ae. aegypti* were selected and subjected to predatory efficiency experiments. As many as 25 larvae were entered into each jar, and the longitudinal observations were made at 5, 10, 30, 60, 120, 240, 360, 480, 600, and 720 minutes. The addition of the next larvae was carried out if the remaining larvae in the jar live 5 until the experiment was stopped at 720 minutes. The predatory efficiency of the larvivorous fish was calculated from the percentage of larvae predated.

Statistical analysis

Data analysis was performed using Statistical Package for Science Solution version 15.0. Kruskal-Wallis and Univariate General Linear Model tests were used to analyze the mean comparisons of predatory efficiency based on species, water temperature ranges, and the interaction of both variables. Statistically, significance in each test used a probability value of $P < 0.05$.

Results

In total, the predation ability of predatory fish differs according to the water temperature level. The average predated larvae at low, optimum, and high-temperature levels were 25.5, 32.8, and 23.4 for *P. reticulata*, 62.3, 75.0, and 49.4 for *B. splendens*, and 59.5, 72.3, and 62.9 for *A. panchax*, respectively. The results of this study showed a decrease in the predation ability of fish at lower and higher temperatures than 27-28 °C, except for *A. panchax* (Table 1). It means that differences in water temperature affected the predation ability of three species of larvivorous fish against *Ae. aegypti* larvae.

A comparison of the predatory efficiency of larvivorous fish at each level of water temperature showed a variation of means and an inter-species significant difference (Table 2). At the low and medium temperatures, *B. splendens* showed the highest predation ability followed by *A. panchax* with a very close average difference, but at the higher temperatures, *A. panchax* was the most voracious compared to the other species. There were two different phenomena of predatory efficiency showed by *B. splendens* and *A. panchax* where both species have a high predation ability. *Betta splendens* has the highest predatory efficiency both in the lower and medium water temperature ranges, while *A. panchax* has the highest predatory efficiency at the high-water temperature only (Fig. 1). This finding showed that *B. splendens* and *A. panchax* are promising larvivorous fish to be exposed to different habitat conditions.

Detailed analysis of the predatory efficiency of the larvivorous fish based on water temperature ranges and exposure time (in four and twelve hours) showed that at the medium water temperature *B. splendens* and *A. panchax* be able to predate 100% of *Ae. aegypti* larvae in four hours, while *P. reticulata* only around 32% (Fig. 1). At the lower range of water temperature, the predatory efficiency of *B. splendens* and *A. panchax* decreased more than 30

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% and 55% in the four hours first although the species can predate 100% of *Ae. aegypti* larvae ten hours later (Fig. 2a, d). At the higher range of water temperature, the three larvivorous fishes showed a significant difference in predatory efficiency, namely 24%, 54%, and 83% for *P. reticulata*, *B. splendens*, and *A. panchax* respectively. Furthermore, *A. panchax* can predate 100% of *Ae. aegypti* larvae in eight hours, while *B. splendens* needs two hours longer. Overall, *P. reticulata* showed the lowest predatory efficiency at all the levels of water temperature, both in the four and twelve hours of exposure time.

Table 1. Inter-temperature range comparisons of predatory efficiency of three larvivorous fish species against *Aedes aegypti* larvae

Larvivorous fish species	Temperature ranges (Celsius degree)	Predatory efficiency				P value
		Minimum	Maximum	Mean	Std deviation	
Poecilia reticulata	20-21	9	48	25.5	12.7	0.042
	27-28	5	60	32.8	16.6	
	34-35	7	47	23.4	13.6	
Betta splendens	20-21	19	100	62.3	29.2	0.002
	27-28	25	100	75.0	28.9	
	34-35	9	100	49.4	30.9	
Aplocheilus panchax	20-21	11	100	59.5	31.1	0.189
	27-28	21	100	72.3	32.2	
	34-35	11	100	62.9	34.7	

2 The predatory efficiency of *Aplocheilus panchax* remains stable with changes in water temperature.

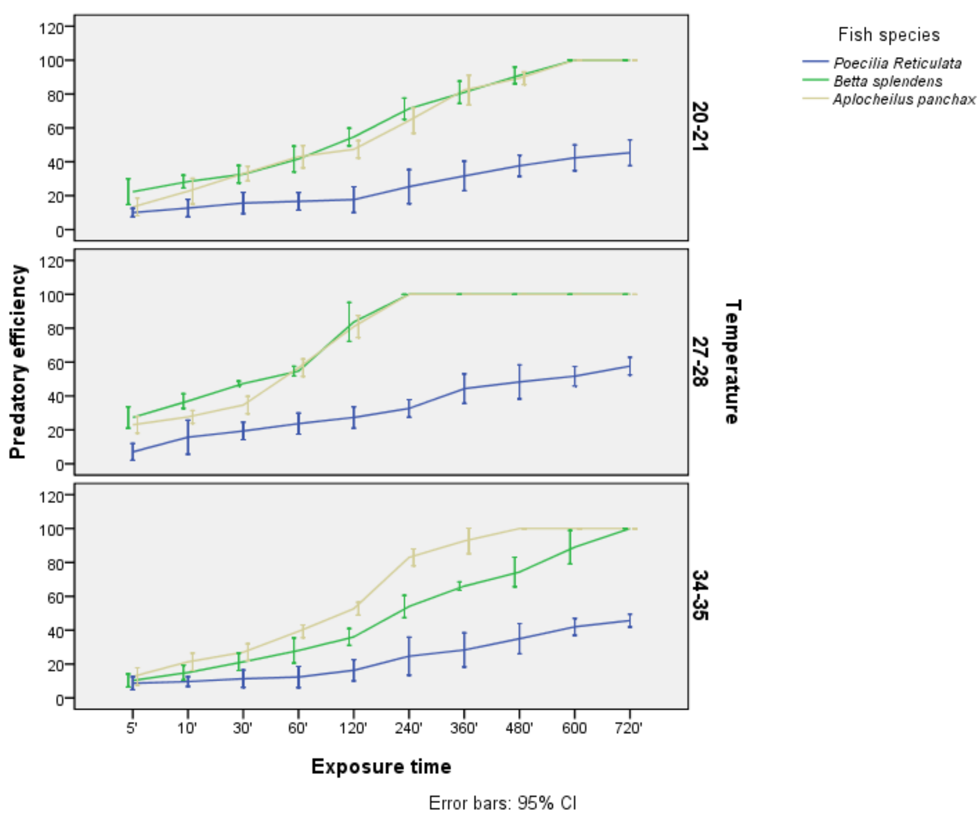

Fig. 1. Predatory efficiency of larvivorous fish based on exposure times (in minutes) and temperature ranges (20–21, 27–28, and 34–35 ºC). *Aplocheilus panchax* showed the fastest and highest predatory efficiency in high-level temperatures

Table 2. Inter-species comparison of predatory efficiency of larvivorous fish in each water temperature range

Temperature ranges (Celsius degree)	Larvivorous fish species	Predatory efficiency				P value
		Minimum	Maximum	Mean	Std. deviation	
20-21	*Poecilia reticulata*	9	48	25.50	12.69	0.000
	Betta splendens	19	100	62.30	29.20	
	Aplocheilus panchax	11	100	59.53	31.48	
27-28	*Poecilia reticulata*	5	60	32.77	16.58	0.000
	Betta splendens	25	100	75.00	28.98	
	Aplocheilus panchax	21	100	72.30	32.18	
34-35	*Poecilia reticulata*	7	47	23.40	13.56	0.000
	Betta splendens	9	100	49.40	30.89	
	Aplocheilus panchax	11	100	62.87	34.70	

The predatory efficiency of the three larvivorous fish species showed significant differences in each temperature range, with close competition between *Betta splendens* and *Aplocheilus panchax*

Discussion

Three larvivorous fish, *P. reticulata*, *B. splendens*, and *A. panchax* have been reported to have a high predatory efficiency against *Ae. aegypti* larvae (9–11) were evaluated in this study. The results showed that the three predatory fish species have different predation abilities and behavior when controlled by water temperature. *Poecilia reticulata* fish have the lowest predation ability at all temperature levels. The most likely reason for this condition is the body size of the fish. Although this variable has been controlled, *P. reticulata* has a shorter body size rather than the other species. The body size of fish represents the bowel length and indicates the predation ability (14). *Betta splendens* fish are the best predators at the medium and the lower-temperature levels, although they are only slightly different from the ability of *A. panchax*. The most important feature of *B. splendens* is the adaptation ability to lower temperature levels. This species has a relatively stable predatory efficiency due to temperature changes from normal to low with a smaller decrease in predation ability rather than *A. panchax*. Both species have an equivalent predation speed. In contrast, *A. panchax* becomes the best and fastest predatory efficiency at the higher temperatures. This finding is consistent with the results of another study where the predation ability of *A. panchax* was higher than *P. reticulata* (15, 20). The other study reported the high predation ability of *A. panchax* against *Aedes* larvae up to 9.2 larvae every five minutes (16) and 463 larvae in 24 hours (17). The reports are consistent with these findings where under low and normal temperature conditions, the predation ability of *A. panchax* reached 30 and 45 larvae in the first 30 minutes of the experiment. The finding should be an important consideration for public health workers in selecting and placing predatory fish species according to their characteristics. *Betta splendens* is suitable for higher altitude areas with low environmental temperatures, whereas *A. panchax* is suitable for lowlands with high environmental temperature levels. Lowlands are more prone to changes in extreme temperatures due to global warming than high-altitude locations (18).

The predatory efficiency of the larvivorous fish was affected by many factors including the fish characteristics, larvae, and environmental conditions. Body size is a fish characteristic that has the most effect on the predatory efficiency of larvivorous fish where this dimension is not only limited to physical appearance but also reflects intestinal length. Different fish species show different body sizes, lengths, and weights. Fish that have longer intestines show more predation ability (14). This physical dimension is related to the sex of the fish where females tend to be bigger and heavier so they can prey on larvae faster and more (19). Larvae characteristics also influence the predation ability of fish, especially the size and movement of larvae. Larvivorous fish tend to prey on smaller larvae, younger instars (20, 21), and larvae that move and salivate with long bursts (22). The most important environmental factors that influence the predatory efficiency of larvivorous fish are pH and temperature, and the presence of other nutritional sources (23). The pH of the water during the experiment showed levels 6–7. *Betta* species including *B. splendens* require optimum conditions at temperatures 22–28 ºC and pH 6.67–7.8 (24, 25) so that in this experiment the fish can show the highest predation ability, even at lower temperatures. The optimum temperature and pH for *P. reticulata* are 29.5–30.5 ºC and 7.84–8.34 (26), and these fish can adapt to extreme low and high temperatures, with lethal temperatures of 10.8–11.8 ºC in low and 38.8–395 ºC in high ranges (27). An increase in water temperature causes these fish to reduce activity and swim slowly near the bottom of the water (28). Extreme acidity (pH), low or high, can interfere with fish physiology. If the pH is higher than 9, the ammonium in the water will be converted to ammoniac forms which are poisoned and can kill fish (29). In addition to the predation aspect, the presence of larvivorous fish in water reservoirs has a deterrent effect on female *Ae. aegypti* mosquitoes to lay their eggs near the surface of the water. The body of the fish secretes kairomone which is easily recognized by female mosquitoes as a sign of danger to avoid laying eggs in that place (30). The limitations of this study have not differentiated predatory efficiency based on the sex and age of the fish, and do not measure oxygen concentrations in water.

This finding reaffirms the important role of larvivorous fish in controlling the dengue vectors in endemic areas where the opened-large size water reservoirs are found. Previous studies have recommended the use of larvivorous fish for the conditions of the areas (6). The use of larvivorous fish is a simple method to eradicate vector mosquitoes by presenting their natural enemies. More specifically, these findings also provide important evidence for the use of larvivorous fish by considering the specific local conditions, especially water temperatures. The selection of suitable fish species can have the advantage of the maximum predation effect.

Conclusion

Two larvivorous species, namely *B. splendens* and *A. panchax* have high predatory efficiency at different temperature levels. Despite the decline, the predatory efficiency of *B. splendens* was the highest at low temperatures and efficient for eradicating mosquito larvae in mountainous water containers, while *A. panchax* was the highest at high temperatures and efficient for eradicating mosquito larvae in hot lowland conditions. This finding is important information for public health workers in selecting larvivorous fishes for the eradication of Dengue vectors in areas that have different environmental temperatures.

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Ethical considerations

Ethical Clearance of this study was obtained from the Ethics Commission of Health Research of Public Health Faculty, Universitas Muhammadiyah Semarang Number: 263/KEPK-FKM/UNIMUS/2019.

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Conflict of interest statement

The authors declare there is no conflict of interests.

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