



## The Influence of Digestive Tract Length of Larvivorous Fish Related to Predation Potential on *Aedes aegypti* Larvae

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### Abstract

**Background :** Biological vector control by using larvivorous fish will be beneficial in reducing *Aedes aegypti* population, hence reducing risk of dengue virus transmission. It is important to select the larvivorous fish according to its digestive organ. Current study aimed to investigate the predation potential among the fish species and to identify the influence of the digestive tract length of the fish related to their predation potential. **Methods :** The research was an analytical observational study with post-test only design. Third stage larvae of *Aedes aegypti* were used as preys for tilapia (*Oreochromis niloticus*), common carper (*Cyprinus carpio*), and guppy (*Poecilia reticulata*). **Results:** In association with their digestive tract length, predation potential of tilapia, common carper, and guppy showed statistical differences ( $P < 0.05$ ). Tilapia demonstrated highest predation of the larvae, followed by common carper and guppy. There are associations between difference in shapes of mouth and intestines, mouth width, intestinal length, and predation potential of these fish species. Current study results showed possible associations between digestive tract length of tilapia, common carper and guppy and predation potential on *Aedes aegypti* larvae, allowing these fish species to be used in biological control of *Aedes aegypti*.

### INTRODUCTION

Dengue Haemorrhagic Fever (DHF) is caused by Dengue viruses and is transmitted by *Aedes aegypti* (WHO, 2012). Currently DHF is still classified as an important disease in public health, as this disease causes high mortality (Bhatt et al., 2013). To overcome and to eliminate DHF, reduction of *Aedes aegypti* population is an efficient and effective program to minimize potential outbreak of DHF (Chang et al., 2011). One of the methods to reduce burden of DHF is by vector control by reducing the number of larval stages of *Aedes aegypti* (Trewin et

al., 2017).

Indonesian Ministry of Public Health suggested a program to overcome the DHF problem called the '3M Plus' (MOH, 2010). This program aims to drain water reservoirs, to bury thrifths that may act as potential water reservoirs, and to cover any kind of water reservoirs. Addition of larvicidal agents into those water reservoirs is also part of the '3M Plus' program. Meanwhile, chemical vector control method by using synthetic chemical insecticides causes negative impact such as development of insecticide resistance and may be potentially harmful to non-

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target populations (Bouزيد et al., 2016). Residue of synthetic chemical insecticides is difficult to degrade, hence it may also cause harm to the ecosystem. Natural insecticides have been recently mentioned as substitutes for synthetic insecticides as these natural insecticides are easily broken down (Sritabutra & Soonwera, 2013). Even so, the insecticidal effects of natural insecticides are generally short, so that the application needs to be repeated frequently (Trewin et al., 2017). One of the alternative methods to reduce mosquito vector of DHF is to use the biological vector control method by using larvivorous fish (Paiva et al., 2014; MOH, 2010).

A study reported that tilapia (*Oreochromis niloticus*) showed good larvae predation potential by being able to feed on 246 mosquito larvae for 24 hours, whereas common carp (*Cyprinus carpio*) could feed on 148 mosquito larvae for 24 hours (Bibi et al., 2017; Sheetal & Shate, 2015). Another study demonstrated that guppy (*Poecilia reticulata*) also showed a good predation potential, as this species could feed on 47 mosquito larvae for 6 hours (Mutmainah et al., 2014). Therefore, tilapia, common carp, and guppy had demonstrated benefit in vector control. These fish species may be chosen as biological vector control in permanent and semi-permanent aquatic habitats. However, problems arose when the water became dirty due to their excretory products. Such water problem can be minimized by selecting proper and efficient species of larvivorous fish.

Current study aimed to investigate the predation potential among the fish species and to identify the influence of the digestive tract length of the fish related to their predation potential. The presence of larvivorous fish in the natural breeding places of mosquitoes triggered multiple interactions and impacts not only to the organisms themselves, but also to the environment. As the association between digestive tract anatomies of larvivorous fish with their predation potential is poorly understood, a study about differences in larvae predation potential of larvivorous fish in association with their digestive tract organs is necessary in order to select appropriate species in biological vector control. Current study results can also be used as supporting data in the policy formulation in DHF vector control.

## METHODS

The research was an analytical observational study with post-test only design. Approval to conduct the research was given from the Faculty of Medicine, Universitas Gadjah Mada (Reference no. UGM/KU/Prst/057/M/05/07). Larvivorous fish species used in the research were tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio*), and

guppy (*Poecilia reticulata*) aged 3-4 weeks. The fish were obtained from local fish market in Yogyakarta, Indonesia and were brought to the laboratory. The body length of tilapia, common carp, and guppy were measured from the tip of the mouth to the end of the body, excluding the caudal fin. Live and healthy 3<sup>rd</sup> stage larvae of *Aedes aegypti* were used as preys to observe the predation potential of larvivorous fish. The research started on 3 April 2014 and ended on 19 June 2014.

The fish were quarantined for one week before the research started to allow matching process: to obtain similar fish condition and stomach contents (Vinson & Angradi, 2011). Environmental temperature and humidity, as well as water temperature were recorded during the study. The fish were fed once daily in the early morning and feeding stopped at 24 hours before the research started in order to empty the fish stomach. Fish were divided into three groups: Group 1, consisted of 5 tilapias; Group 2, consisted of 5 common carpers; and Group 3, consisted of 5 guppies. Each fish in each group was put in one tank (diameter of 10 cm and depth of 15 cm, filled with 1 l of water), and into each tank was given 200 3<sup>rd</sup> stage larvae of *Aedes aegypti*. The reduction in number of larvae was noted at 1 hour, 6 hour, 12 hour, 18 hour, and 24 hour. Interventions in each subgroup were performed in 3 replications. Reduction in number of larvae, or predation potential, was calculated from the following formula (Cowan and Houde 1993):

$$P = ((U-M))/n$$

P = reduction in number of larvae (predation potential)

U = total number of larvae fed by larvivorous fish

M = number of remaining larvae

n = number of larvivorous fish tested

Data analysis was performed using SPSS ver.17 (SPSS Inc., USA). Feeding rate (predation potential) was analyzed by using one-way ANOVA and Least Significant Difference (LSD) analysis in order to compare the feeding rate on *Aedes aegypti* larvae of tilapia, common carp, and guppy with the density of larvae. The predation potential was analyzed from data obtained in 1 hour, 6 hour, 12 hour, 18 hour, and 24 hour. Level of significance used for statistical analysis was  $P < 0.05$ .

## RESULTS AND DISCUSSION

In current study, tilapia had average body length of  $2.68 \pm 0.13$  cm, whereas common carp had average body length of  $2.50 \pm 0.16$  cm, and guppy had average body length of  $2.53 \pm 0.15$  cm. Tilapia showed the highest average of body length, body

Table 1. Characteristics of body length, body weight, mouth width, larvae-mouth width ratio, and intestinal length of tilapia (*Oreochromis niloticus*), common carper (*Cyprinus carpio*), and guppy (*Poecillia reticulata*).

Character-istics	Unit	Tilapia ( <i>Oreochromis niloticus</i> )*	Common carper ( <i>Cyprinus carpio</i> )*	Guppy ( <i>Poecillia reticulata</i> )*
Body length	cm	2.68 ± 0.13	2.50 ± 0.16	2.53 ± 0.15
Body weight	g	0.62 ± 0.04	0.29 ± 0.06	0.28 ± 0.04
Mouth width	cm	0.04 ± 0.3	0.1 ± 0.08	0.25 ± 0.05
Larvae-mouth width ratio	larvae/cm	390 ± 23.6	1,022 ± 49.7	237 ± 45.1
Intestinal length	cm	10.37 ± 1.08	3.28 ± 0.75	4.27 ± 0.38

\*shown in mean ± SD

weight, and intestinal length compared to common carper and guppy (Table 1).

On the other hand, common carper demonstrated the highest average of mouth width and larvae-mouth width ratio compared to the other two species. However, none of these differences demonstrated statistical significance. Macroscopic appearances of tilapia, common carper, and guppy used in the study were illustrated in Figure 1. The average environment humidity during the research was  $74.44 \pm 0.72\%$ , while average water temperature was  $27.5 \pm 0.43^\circ\text{C}$ .

Larvae density reduction of *Aedes aegypti* larvae (predation potential) in current study showed difference on each tilapia, common carper, and guppy in each period of observation. Feeding observation time at 1 hour showed that tilapia could feed more on *Aedes aegypti* larvae compared to common carper and guppy (Table 2).

There was a statistically significant difference ( $p < 0.05$ ) observed in predation among the fish species at 1 hour of observation. As described in Table 2, predation potential of tilapia showed a dramatic decrease at 6 hours of feeding observation time. Predation potential by common carper and guppy showed decrease as well. Statistical significance was observed in the predation difference ( $p < 0.05$ ).

On the other hand, feeding observation time at 12 hours showed that tilapia and common carper revealed similar predation potential, whereas guppy demonstrated lower predation potential. However, the predation potential among the three species did not show significant difference. At 18 hours, both common carper and guppy showed decreasing ability in predation. Interestingly, tilapia demonstrated increasing predation potential compared to that of previous observation time (Table 2). These differences were statistically significant ( $P < 0.05$ ). Feeding

observation time at 24 hour showed that tilapia had reduced predation on *Aedes aegypti* larvae, while common carper and guppy exhibited constant predation potential. The predation differences among three fish species have proven statistical significance.

In general, predation potential analyzed using LSD method showed that average feeding rate of guppy showed lower results compared to those of tilapia and common carper. Tilapia demonstrated highest average feeding rate on 3<sup>rd</sup> stage larvae of *Aedes aegypti* at all observation time.

Natural characteristics of larvivorous fish to feed on preys is essential to be applied as biological vector control agent to reduce the population of DHF vector. Mosquito predators as a biological control for the aquatic larval stage of mosquitoes. Parallel to current study results, several other studies showed that tilapia demonstrated promising predation potential against *Aedes aegypti* and *Culex quinquefasciatus* larvae (Bibi et al., 2017; Louca et al., 2009; Benelli et al., 2016). Common carp and guppies have also been reported to be natural biological vector control against *Aedes aegypti*, *Aedes albopictus* or *Culex quinquefasciatus* (Sanyal & Ghosh, 2014; Saleeza et al., 2014; Londhe & Sathe, 2015).

Predation potential in association with sex of tilapia, common carper, and guppy was not studied, as it was difficult to distinguish sex based on the morphology of younger fish. Rebenburg mentioned that sex of fish did not influence fish behavior, and that feeding behavior of fish correlated with sensory stimulation to the preys (Rebenburg, 2010). Predation potential of fish is the capability of fish to feed on preys. In current research, 3<sup>rd</sup> stage larvae of *Aedes aegypti* were used as natural preys for tilapia, common carper, and guppy. Daily food intake of fish depends on their aggressiveness. Smaller fish may act more aggressively than the big ones

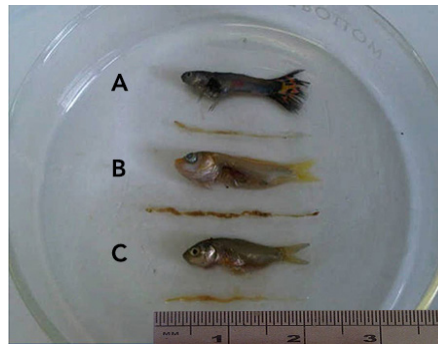


Figure 1. Comparison of macroscopic appearances of guppy (A), tilapia (B), and common carper (C).

to get food. Additionally, younger fish seek for food more frequently than mature fish in order to meet their growth demand (Lall & Tibbetts, 2009; Martin et al., 2010; Sahtout et al., 2018; Manon & Hossain, 2011) growth, and efficiency in juvenile tilapia (*Oreochromis niloticus*).

Smaller fish demonstrated lower predation potential compared to that of bigger fish (Novaes & Carvalho, 2012). Mini tanks used in current study were possibly too small, containing only 1 l of water, hence easier for smaller fish to feed on preys than bigger fish. Additionally, it was easier for the fish in current study to feed on the larvae due to frequent visual of larvae, as visual stimulation influences feeding behavior of fish (Rebensburg, 2010).

Size of larvae may have impact on the amount

of larvae fed by fish. In regard to mouth width and stomach size, containment of mature, bigger larvae will be more difficult (Liew et al., 2012). Commonly, fish that are always feed on tiny preys usually have small stomach. Several species of fish, such as tilapia, can expand their stomach size, allowing them to feed on more preys. However, increasing frequency of feeding will reduce the aggressiveness of fish, hence reducing the amount of preys fed (Vinson & Angradi, 2011; Sahtout et al., 2018; Shafi et al., 2012). This is demonstrated in current study results, that the numbers of larvae ingested by fish were generally decreasing over time.

Compared to those of common carper and guppy, current study demonstrated that tilapia had bigger size in body length, body weight, and intes-

Table 2. LSD of feeding rate of tilapia (*Oreochromis niloticus*), common carper (*Cyprinus carpio*), and guppy (*Poecillia reticulata*) as predators of *Aedes aegypti* observed at 1 hour, 6 hour, 12 hour, 18 hour, and 24 hour.

Time	Species	Average predation	LSD ( $\alpha=0.05$ )
1 Hour	<i>Oreochromis niloticus</i>	114 <sup>b</sup>	72.94
	<i>Cyprinus carpio</i>	40 <sup>a</sup>	
	<i>Poecillia reticulata</i>	36 <sup>a</sup>	
6 Hours	<i>Oreochromis niloticus</i>	19 <sup>a</sup>	17.91
	<i>Cyprinus carpio</i>	18 <sup>a</sup>	
	<i>Poecillia reticulata</i>	12 <sup>a</sup>	
12 Hours	<i>Oreochromis niloticus</i>	21 <sup>b</sup>	15.91
	<i>Cyprinus carpio</i>	20 <sup>b</sup>	
	<i>Poecillia reticulata</i>	4 <sup>a</sup>	
18 Hours	<i>Oreochromis niloticus</i>	47 <sup>b</sup>	10.66
	<i>Cyprinus carpio</i>	7 <sup>a</sup>	
	<i>Poecillia reticulata</i>	3 <sup>a</sup>	
24 Hours	<i>Oreochromis niloticus</i>	28 <sup>b</sup>	7.17
	<i>Cyprinus carpio</i>	7 <sup>a</sup>	
	<i>Poecillia reticulata</i>	3 <sup>a</sup>	

a-a :  $I(x_1 - x_2)I \leq LSD\alpha$  = not significantly different

a-b :  $I(x_1 - x_2)I \geq LSD\alpha$  = significantly different



tinal length. This might explain higher predation potential of tilapia contrasted to other fish species. Bigger body size and larger stomach may contain more preys (Khojasteh, 2012, Hernandez et al., 2009; Raji & Norouzi, 2010). Additionally, tilapia showed significantly higher number of larvae ingested during the first few hours of observation. As the stomach filled, the feeding rate decreased in all species.

Fish show rhythms of feeding and select their food as well. Fish seek for food according to their feeding rhythms at certain times of the day: at daylight or at nighttime. Fish show an inter-specific and intra-specific interaction regarding feeding behavior in each phase of their lives. In general, tilapia, common carper, and guppy feed on preys at daylight. However, current study results demonstrated that at each time of observation, the number of larvae has always been reduced. This showed flexibility of feeding rhythm, a good characteristic as biological vector control agents. Feeding behavior of fish resulted from responses to environment factors, including light, water condition, amount of preys, temperature, dissolved oxygen, and combinations of these factors (Luchiari & Freire, 2009; Carvalho et al., 2013; Novaes & Carvalho, 2012)

A proper selection and application of larvivorous fish as biological vector control to reduce the population of *Aedes aegypti* should be further introduced to society in order to reduce and to control DHF vector. According to current research results, tilapia (*Oreochromis niloticus*) demonstrated promising results as the agent for biological vector control program.

## CONCLUSION

There were significant differences in predation potential of tilapia, common carper and guppy against 3<sup>rd</sup> stage larvae of *Aedes aegypti*. Measurements of digestive tract length showed differences between tilapia, common carper, and guppy. There were possible associations between digestive tract length of tilapia, common carper and guppy and predation potential on *Aedes aegypti* larvae.

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