GUPPIES AS PREDATORS OF COMMON MOSQUITO LARVAE IN MALAYSIA

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Abstract. Observation on predation activities of guppies (*Poecilia reticulata*) on the larvae of three species of mosquito, namely *Aedes albopictus*, *Aedes aegypti*, and *Culex quinquefasciatus* was carried out under laboratory conditions. Male and female guppies were used as predators for predation experiments on the 4th instars of mosquito larvae. The daily feeding rates comparing male and female guppies on mosquito larvae were different; the female guppies consumed more mosquito larvae than male guppies did. The daily feeding rates of female guppies were 121.3 for *Ae. aegypti*, 105.6 for *Ae. albopictus*, and 72.3 for *Cx. quinquefasciatus*. The daily feeding rates of male guppies were 98.6 for *Ae. aegypti*, 73.6 for *Ae. albopictus*, and 47.6 for *Cx. quinquefasciatus*. In terms of prey preference, there was greater preference towards mosquito larvae of *Ae. aegypti*, followed by *Ae. albopictus*, and the least preferred was *Cx. quinquefasciatus*. Male and female guppies consumed more mosquito larvae during lights on (day time) compared with lights off (night time). The water volume, prey species, number of fish predators available, prey densities, and prey's sex also influenced the predation activities.

Keywords: *Ae. aegypti*, *Ae. albopictus*, *Culex quinquefasciatus*, guppy, predation activities, Malaysia

INTRODUCTION

Natural predators are used in biological control. This type of control is an environmentally friendly preventative method to control, among others, populations of pest organisms (Becker, 2006). Several organisms have proved to be effective predators against mosquito larvae, such as larvivorous fish, mosquito of the genus *Toxorhynchites*, dragonflies and damselflies, cyclopoid copepods, nematode, *Bacillus thuringiensis* H-14, and *B. sphaericus* (WHO, 1986). Biological control of mosquitoes was very popular during the early part of the 20th century, but this type of control has been replaced with the insecticidal control due to easy availability of chemicals such as organochlorines and organophosphates. However, because of problems with insecticide resistance and greater awareness of environmental contamination, there has been renewed interest in biological methods (Service, 2000).

Among the popular biocontrol agents against mosquito populations are the larvivorous fish, or mosquito fish: *Gambusia affinis* and *G. holbrooki*. This species, however, is ineffective for the control of

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mosquitoes in what is termed as "container habitats", that is, mosquito breeding places such as phytotelmata, tree holes, bamboo nodes, and discarded containers (Kumar and Hwang, 2006). Another commonly used fish species for biocontrol is the South African guppy, *Poecilia reticulata*, which can tolerate waters of high temperatures or that are organically polluted. Carps, *Cyprinus carpio*, found in Chinese rice fields are used to control *Aedes aegypti*. Edible catfish, *Clarias fuscus* in Myanmar, *Oreochromis*, Tilapia species in Africa, and *Aplocheilus* species in Europe and Asia are placed in water storage tanks to control *Ae. aegypti* (Service, 2000). Additionally, guppies of the *Poecilia reticulata* species are used to control dengue vector, *Ae. aegypti*, in domestic water storage containers in rural areas of Cambodia (Seng *et al*, 2008). In Kenya, the use of fish as biocontrol agents has been proved effective towards malaria mosquito larvae (Howard *et al*, 2007; Kweka *et al*, 2011). *P. reticulata* also shows preference for other prey besides mosquito larvae (Manna *et al*, 2008).In this study, the efficacy of the natural predator, *P. reticulata* was investigated using mosquito larvae of *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* as prey.

MATERIALS AND METHODS

The guppies, *P. reticulata*, used as predators in the experiments were collected in the drains of Putrajaya and Kuala Selangor districts. All fish were recorded for their wet weights and lengths before and after the experiment. Before the start of the experiment, all the fish used were acclimatized to laboratory conditions and were placed in plastic aquaria for a period of one week prior to the actual date of experimentation. All fish were provided with bloodworm and fish food as a diet for maintenance. Guppies were starved for 24 hours before introduction to the experimental aquaria, as this period would increase the motivation to feed (Bhattacharjee, 2009).

The experimental aquaria contained pond water for the feeding efficacy experimentation. For this experiment, the daily feeding rates of guppies for three species of mosquito larvae were recorded. The *P. reticulata* was exposed to 100 4^{th} instar larvae for each species of *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus.* Three aquaria were arranged for each mosquito species, and three replicates of experiments were completed on separate days. The time taken for the first attack of guppies on first mosquito larvae was recorded and the daily feeding rate was recorded repeatedly at 3-hour interval.

The same mosquito larvae and fish were not used in subsequent experiments. At each 3-hour interval, the water from experimental aquaria was sieved and transferred to a white tray to count the number of mosquito larvae consumed by the predator fish. The experiment was carried out within 24 hours from 5:00 AM until 5:00 PM for 'lights on' and from 5:00 PM until 5:00 AM for 'lights off'. Using this experimental procedure, the active periods of feeding behavior for the fish, *P. reticulata* could be obtained.

The second experimental setup was to assess the relationship of feeding rate with different water volumes together with the number of predator and prey densities. This procedure was done in three replicates for each species the experimental protocols used are: 1) Aquaria A female fish (1x1x100): single fish with 1 liter of water volume and 100 4^{th} instar of mosquito larvae; 2) Aquaria B female fish (1x2 x100): single fish with 2 liters of water volume and 100 4th instar of mosquito larvae; 3) Aquaria C female fish (2x1x100): two fish with 1 liter of water volume and 100 4th instar of mosquito larvae; 4) Aquaria D female fish (1x1x200): single fish with 1 liter of water volume and 200 4th instar of mosquito larvae; 5) Aquaria A male fish (1x 1x100): single fish with 1 liter of water volume and 100 4th instar of mosquito larvae; 6) Aquaria B male fish (1x 2x100): single fish with 2 liters of water volume and 100 4th instar of mosquito larvae; 7) Aquaria C male fish (2x 1x100): two fish with 1 liter of water volume and 100 4th instar of mosquito larvae; 8) Aquaria D male fish (1x 1x200): single fish with 1 liter of water volume and 200 4th instar of mosquito larvae.

The data of daily consumption rate of both male and female guppies were analyzed using one-way ANOVA (SPSS®; IBM, Armonk, NY), and the relationships between consumption rate and variation of water volume, prey species, number of fishes, prey densities, and sex were analyzed using multiple regression.

RESULTS

Exposing 100 4th instar mosquito larvae of every species to a single predator species assessed the daily feeding rate. The number of larvae remaining was recorded at every 3-hour interval, and the experiment was carried out over 24 hours.

The overall feeding rates of female guppies were significantly higher than males for all 3 species of mosquitoes tested. Both female and male guppies showed greater preference for *Ae. aegypti* larvae, followed by *Ae. albopictus*, and the least preferred was *Cx. quinquefasciatus* (Fig 1). It was observed that the female guppies were more aggressive than male guppies as they consumed more mosquito larvae species. Both male and female guppies spent most of their time at surface water and were active in searching mosquito larvae, but the female guppies were more aggressive than male guppies. When the mosquito larvae were released in the aquaria, the first attack of guppy was very fast.

The feeding rate between lights on and lights off also varied between male and female guppies, but both were active during lights on. As shown in Fig 2, both predators were active during lights on as they consumed more mosquito larvae during this time. The presence of light influenced the feeding rate as the predator can easily search and attack the prey. Some studies indicate that when the water is turbid, fish find it difficult to search for prey because their vision is not clear (Robertis *et al*, 2003; Turesson and Brönmark, 2007; Jacobsen *et al*, 2014)

In this study, 100 and 200 4th instars of mosquito larvae were used in the predation experiment. When 100 4th instars of mosquito larvae were exposed to the guppies, they consumed all mosquitoes, and when 200 4th instars of mosquito larvae were exposed to the guppies, more than 100 4th instars were consumed. Larvae consumption increased, as there was an increase in prey densities, until satiation level was reached (Table 1).

From the regression equation, it was observed that the feeding rate of male guppies increased when water volume was increased, and the number of predators was increased. Conversely, the feeding rate of female guppies increased when water volume and mosquito larvae densities were increased. All the three factors influenced the predation activities of both male and female guppies.

Table 1
Feeding rate of male and female guppies in 24 hours (mean ± SE of 3 experiments) on larvae of *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus* with the variations in mosquito species, water volume, number of fish, and mosquito larvae densities.

Sex of guppies	Mosquito species	Water volume	Fish (*n*)	Mosquito densities	Mean ± SE
Male	*Ae. albopictus*	1	1	100	49.67 ± 1.202
		1	2	100	100.00 ± 0.000
		1	1	200	53.00 ± 2.887
		2	1	100	37.33 ± 1.453
Female	*Ae. albopictus*	1	1	100	87.00 ± 7.572
		1	2	100	100.00 ± 0.000
		1	1	200	102.00 ± 10.599
		2	1	100	73.00 ± 5.686
Male	*Ae. aegypti*	1	1	100	77.00 ± 9.815
		1	2	100	100.00 ± 0.000
		1	1	200	101.00 ± 2.082
		2	1	100	60.00 ± 4.726
Female	*Ae. aegypti*	1	1	100	100.00 ± 0.000
		1	2	100	100.00 ± 0.000
		1	1	200	123.00 ± 4.619
		2	1	100	85.00 ± 2.887
Male	*Cx. quinquefasciatus*	1	1	100	46.00 ± 5.196
		1	2	100	100.00 ± 0.000
		1	1	200	50.00 ± 8.544
		2	1	100	34.00 ± 6.083
Female	*Cx. quinquefasciatus*	1	1	100	77.00 ± 6.928
		1	2	100	85.00 ± 4.041
		1	1	200	94.00 ± 6.351
		2	1	100	65.00 ± 4.041

Table 2
Coefficients provided by multiple regression analyses for predation of mosquito larvae by guppies.

Sex of guppies	Equations for larval feeding rate	R-value
Male guppy	$Y = 44.55 - 19.0\ X1 + 37.22\ X2$	0.77
Female guppy	$Y = 93.83 + 17.16\ X1 + 14.83\ X2$	0.67

Y, feeding rate of larvae; X1, water volume; X2, number of predator; R, multiple correlation coefficient. For female guppy X2, prey densities (mosquito larvae densities).

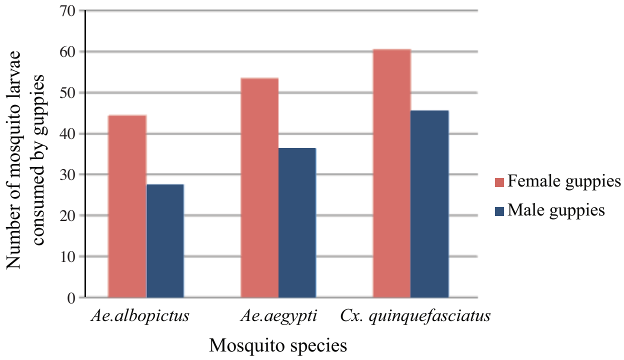

Fig 1–Number of mosquito larvae consumed by female and male guppies (*P. reticulata*) on *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus* larvae.

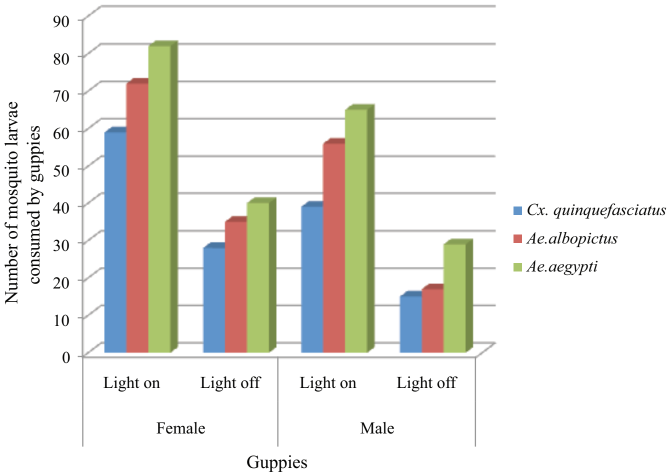

Fig 2–Feeding rate of female and male guppies (*P. reticulata*) on *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus* larvae in 24 hours during light on and light off.

DISCUSSION

In this study, guppies were used as predators against three common mosquito larvae in Malaysia. Saleeza *et al* (2011) reported that the three common mosquito larvae, *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus*, are commonly found in residential areas in both urban and sub-urban areas. A number of studies have indicated that guppies of the *P. reticulata* species are good predators, as they can control mosquito larvae population (Manna *et al*, 2008; Seng *et al*, 2008; Anogwih and Makanjuola, 2010; Ghosh *et al*, 2011). However, guppies do not choose *Cx. quinquefasciatus* when other available foods are present in polluted water or drain water, such as plankton (Dua *et al*, 2007). *P. reticulata* is a more effective predator against *Ae. albopictus* larvae compared to other fish species such as *Puntius bimaculatus* and *Rasbora caverii*. A study in Sri Lanka suggested that, in comparison to *Toxorhynchites* larvae, which are also larvae predators, the fish consumed more mosquito larvae (Wijesinghe *et al*, 2009).

In this study, it was observed that female guppies (*P. reticulata*) ate mosquito larvae more than male guppies did. This result supports the findings of Seng *et al* (2008): female guppies ate more than male guppies, with 122.9 and 74 *Ae. aegypti* larvae per day, respectively. This is due to the larger size of the female guppies. Therefore, female guppies can consume more mosquito larvae than male guppies can.

The number of prey consumed varies with the difference in body size. This means that prey consumption increases with body size (Manna *et al*, 2008). This result also supports the finding by Cavalcanti *et al* (2007) who report that the efficacy as predator depends on its weight and sex. Cavalcanti *et al* (2007) used five different fish species as predators against

Ae. aegypti larvae and found that the larger fish are more effective predators, and female guppies are more capable to eradicate *Ae. aegypti* larvae than male guppies are.

According to Neng *et al* (1987), the predation efficiency of *Clarias fuscus* depends on the body weight of the predator. Therefore, larger fish eat more mosquito larvae than smaller fish do. According to Anyaele and Obembe (2010), the adult female fish is more voracious and has higher biocontrol potential compared to the adult male fish. Moreover, in terms of feeding rate, the daily feeding rate of the guppies on mosquito larvae species was different between sexes. However, the prey preferences of both sexes of guppy were the same, as they preferred *Ae. aegypti* > *Ae. albopictus* > *Cx. quinquefasciatus* larvae, and they tried to avoid the pupa stage of mosquito development.

Most frequently, male and female guppies were at the water surface actively searching and attacking mosquito larvae; however, they totally avoided mosquito pupae. This behavior has also been observed by Anogwih and Makanjuola (2010). Moreover, in this study, it was observed that male and female guppies were more active and consumed more mosquito larvae during lights on period. Anyaele and Obembe (2010) also found that fish eat more under lights on compared with dark time, and they suggest that fish rely on their visual ability to search for prey. However, according to Ghosh *et al* (2011), this difference in eating behavior during daytime and nighttime had no practical significance in a biocontrol strategy.

In terms of prey preference among the three species of mosquito larvae, both sexes of guppy prefer *Ae. aegypti* larvae compared with the other two species of mosquito larvae. Factors that influence the selectivity of prey by predator are as follows: how the prey escapes from the predator (guppy), ability of the predator, prey attraction, and posture of mosquito larvae. Deacon (2010) states that the factors that influence the predation activities are vegetation, feeding behavior of different mosquito genera, ability to escape, distinct morphology, posture in the water for mosquito larvae, and color of the larvae.

In this study, both sexes of guppy preferred *Ae. aegypti* larvae compared with *Ae. albopictus* and *Cx. quinquefasciatus* larvae. As suggested by Kesavaraju *et al* (2007), the predation rate is influenced by the behavior of prey species and development stage of prey. This means that different predator attacks different stage of mosquito larvae. However, in this study, the 3rd and 4th instar larvae used as the trial experiment showed that guppy preferred late-stage larvae compared with early-stage larvae. The black color of the *Ae. aegypti* larvae could be the factor that attracted the guppy to attack and consume them, unlike the *Cx. quinquefasciatus* larvae that are pale in color.

Otherwise, Rajasekharan and Chowdaiah (1972) suggest that the preference of Gambusia for *Ae. aegypti* larvae could be attributed to the larvae's small size, their vertical position in water, and their tendency to clump in groups; these factors facilitate their capture. Kar and Aditya (2003) state that planaria prefer and consume *Anopheles* larvae more than they do *Culex* larvae. It is likely due to the behavior of the prey and the predator, as the larval posture of *Anopheles* larvae is parallel to the water surface. This posture helps planaria to attack the larvae more easily.

Additionally, Kar and Aditya (2003) state that *Culex* larvae are more active and move faster than *Anopheles* larvae and *Culex* larvae are more difficult to be attacked. This suggests a reason that both sexes of guppy observed in this study found difficulty to attack and consume *Cx. quinquefasciatus* compared with the other two species. Anyaele and Obembe (2011) found that larvivorous fish prefer *Anopheles* compared with *Culex*. It is most likely because *Culex* has the ability to escape faster than *Anopheles*.

As reported by Culler and Lamp (2009), the preference towards certain type of prey is not only due to the ease to capture they prey, but it also depends on availability and profitability to the predator. For example, although ostracods are easy to capture, they lack the nutritional composition needed by predators for their growth, and so predators do not prefer to eat the ostracods (Culler and Lamp, 2009). Anogwih and Makanjuola (2010) also found that guppies prefer alternative prey, that is, *Chironomus* larvae, which are the most preferred prey, only then followed by mosquito larvae and worm larvae.

Manna *et al* (2008) also indicated that guppies prefer alternative preys such as tubificid larvae when this alternative preys are present, but nonetheless guppies still consume mosquito larvae. Manna *et al* (2008) also mentioned that guppies have a wide range of dietary choices. Both studies, Anogwih and Makanjuola (2010) and Manna *et al* (2008), showed that the guppy prefers alternative prey than mosquito larvae when both are present together; however, in both experiment, it was observed that the guppy also consumed the mosquito larvae. Other larvivorous fish, *Aphyosemion gularis* prefers mosquito larvae than non-mosquito macroinvertebrates, such as chironomid larvae (Anyaele and Obembe, 2011).

The factors that influence predation activities have been discussed by Griffin and Knight (2012), and these factors are categorized into two, namely, ecological factors and behavioral factors. The ecological factors include suitable breeding sites or habitat for predator and prey, prey preference by predators, and developmental stage of both prey and predator. The behavioral factors are, for example, the feeding habits of their predator and preference for alternative prey.

The effective way to use biocontrol agents depends on the suitability of the breeding site for predators to eradicate the mosquito population and species preference for mosquito larvae. For example, a study in French Polynesia found that covered sites are preferred by *Aedes* spp and are suitable for *Mesocyclops aspericornis* but not suitable for fish due to insufficient light. Therefore, the most effective way to control *Aedes* spp in covered sites is by using *M. aspericornis.*

The advantage of using fish as a biocontrol agent is that fish has a good adaptation to its new environment (Lardeux, 1992). According to Kweka *et al* (2011), besides habitat type, predator species can also influence predation rate. They found that *Gambusia affinis* is the most effective predator than other predators such as backswimmer, dragonfly nymph, belostomatidae, and tadpoles in consuming *Anopheles gambiae* larvae. Other factors that influence predation activities are number of predators, prey densities, water volume, and respective sizes of predator and prey (Aditya *et al*, 2007; Chandra *et al*, 2008), aquatic vegetation (Savino and Stein, 1989; Shaalan *et al*, 2007), body size of predator, behavior of predator, and mechanism of prey capture (Tranchida *et al*, 2009; Ohba and Takagi, 2010).

In this study, when two fish were exposed to prey, the number of prey consumed was more than when only one fish was released. As a result, more mosquito larvae were consumed by these two fish. This was observed especially when two male guppies were released in the aquaria, but this was not observed when two female guppies were released. It was likely due to competition when the two fish were doing their predation activities. Anogwih and Makanjuola (2010) reported low foraging behavior of guppies when a single fish is exposed to the mosquito larvae. However, when two fish are exposed to mosquito larvae, competition between the two fish was present, thus increasing their foraging behavior.

In terms of feeding rate in this study, female guppies had their feeding rate increased when the prey densities were increased. This result supports the findings of Anyaele and Obembe (2010). They reported that larval consumption increased when the densities of prey increase until satiation level is reached, that is, when the fish becomes overwhelmed. Both species of *Pseudomugil signifer* Kner and *Gambusia holbrooki* Girard consume more larvae at the lowest densities compared to the highest densities. However, both species reached a level of satiation when they are exposed to high densities of larvae and late instars of mosquito larvae (Willems *et al*, 2005).

Water volume also influences predation activities and feeding rate. When 2 liters of water was used in the experiment, the predation activities and feeding rate decreased. Fish spent more time foraging and searching for mosquito larvae. The feeding rate decreased when water volume of water was increased, and the feeding rate increased when the number of predators and the densities of preys were increased (Chandra *et al*, 2008; Mandal *et al*, 2008). As discussed by Jacob *et al* (1983), environmental factors such as temperature and lighting also influence the predation efficiency of larvivorous fish, but salinities do not affect the predation activities. The predation activities increase when the temperature is increased and the feeding rate under lighting is higher than in dark condition. In addition, Marti *et al* (2006) suggested that different prey attack strategies and handling time of predator to consume prey also influence feeding rate.

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