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Research Article

PREY SELECTIVITY AND EFFICIENT BIOCONTROL OF DENGUE BY GUPPIES: EFFECTS OF ALTERNATIVE PREY AND HABITAT COMPLEXITY

S. Sanyal* and S. Ghosh

Post Graduate Department of Zoology, Bethune College, 181 Bidhan Sarani, Kolkata 700006, West Bengal, India

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ABSTRACT

This paper focuses on the foraging behaviour of guppy, *Poecilia reticulata* (Peters, 1859), in the presence of alternative prey, for dengue control efficacy. Predatory potentiality of the fish was studied in a three prey system under different habitat conditions alone or along with two and four conspecifics. The number of *Aedes* larvae consumed in comparison to other prey types (tubificid worms and vegetables) was noted and an index of prey selectivity was used to evaluate the preference for *Aedes* larvae. Foraging experiments were attempted in the laboratory. The study revealed a strong and consistent preference to tubificid worms (selectivity coefficients >0.33, $t_{(5)}$= 8.08; p < 0.001) in a simple habitat three-prey system. However, mosquito larvae (selectivity coefficients >0.33, $t_{(5)}$= 6.74; p < 0.01) were consumed readily when habitat complexity increased. Presence of conspecifics affected male foraging behaviour similarly with *Aedes* larvae as preferred prey in complex habitat and tubificid larvae in simple habitat. Significant difference of niche breadth ($t_{(17)}$ = 5.92; p < 0.001) between simple and complex habitat reflecting shift of prey selection pattern by fish. Consumption rate of different prey types varied with the predator densities in both habitats. The present study would justify the interaction between social and habitat factors affected prey preference and have important implications for the efficacy of poeciliids in bio control of dengue. However, field studies including other prey species will be required to substantiate this finding.

Key words: Alternative prey, Bio control, Dengue, Habitat, *Poecilia reticulata*, Social factors.

INTRODUCTION

Dengue is a disease of increasing public health importance (Gubler, 1998). The disease is transmitted to humans through the bites of female *Aedes (*Yellow fever mosquito, (Insecta: Diptera: Culicidae) mosquitoes (Linneaus, 1774; Skuse, 1893). Over 100 tropical countries are endemic for dengue and report increased epidemics, including more of the severe form of the disease, dengue haemorrhagic fever (DHF) (WHA, 1993; WHO, 1995). In India, DHF was first reported in Kolkata in 1963-64 (Aikat *et al*., 1964). Recently there was a major outbreak in 2012 involving several districts of West Bengal (Bandyopadhyay *et al*., 2013).

Use of synthetic pyrethroids has always given top most priority for mosquito control and prevention (Hargreaves *et al*., 2000). Development of strong form of insecticide resistance stimulated interest in alternative control methods like biological control and biopesticides (Howard *et al*., 2007). The usefulness of fishes in mosquito control is a well-known simple sustainable method for more than 100 years (Fletcher *et al*., 1992). The larvivorous fish *Poecilia reticulata*, a native of South America, is used as an effective biological control agent of mosquitoes in different habitats in Australia, India, particularly in Kolkata (Hati and Saha, 1989, 1994; Lindholm *et al*., 2005).

The selection of a biological agent should be based on its self replicating capacity, preference for the target pest population in the presence of alternate natural prey, adaptability to the

*Corresponding author e-mail: sutapa2007.sanyal@gmail.com

introduced environment, and overall interaction with indigenous organisms (Arthington and Marshall 1999; Denoth *et al*., 2002; Carlson *et al*., 2004). Assessment of mosquito preference is a pre-requisite to promote a fish species for biological control (Deacon, 2010). Potential fish predators failing to show adequate selectivity for mosquito amongst wide range of prey can influence the stability and diversity of the wetland community (Blaustein and Chase, 2007). The effectiveness of larvivorous fish to control mosquitoes may vary due to environmental complexity (Chandra *et al*., 2008). It has to be kept in mind that aquatic vegetation can interfere with fish feeding and can also provide refuge for the mosquito larvae (Chandra *et al*., 2008).

Sharp declines in the number of malarial cases were noticed in India after introduction of effective biocontrol procedures with *Poecilia reticulata* (Sharma and Ghosh, 1994; Ghosh *et al*., 2005 and Ghosh and Dash, 2007, 2009). However, reports on biological control of *Aedes* mosquitoes employing *Poecilia reticulata* are limited. Few studies have examined guppy foraging in the presence of alternative prey and the effects of social and physical aspects of the foraging environment.

The present paper describes the predatory efficacy of *Poecilia reticulata* against *Aedes* larvae under laboratory conditions using multiple alternative prey and habitat conditions.

MATERIALS AND METHOD

Collection and maintenance of predators

Guppies were collected from the local sewage drains of North Kolkata of the state of West Bengal, India. The fishes were 3.1-4.0 cm in length and 0.26-0.38 g in weight. They were acclimatized for seven days in the laboratory in holding aquarium containing dechlorinated water. Fish were kept in a temperature of 25 to 30°C and were fed with fish food (Tokyu®, Tokyo Corp., Japan) before using them in experiment. In all the experiments, individual fishes were starved for a period of 24 hrs before introduction into the experimental aquaria.

Collection and maintenance of prey

Aedes larvae were collected from earthen flower pots and discarded containers of the Bethune College campus, Kolkata, West Bengal, India. Most *Aedes* larvae can be distinguished from other genera by the unaided eye by their short siphon (Nelson, 1986). The larvae were selected to be approximately the same size and all were early 4^{th} instar.

In the laboratory, the collected larvae were emptied in enamel trays to segregate larger one (4^{th} instar) based on body length. The collected small larvae were placed in separate containers. The smaller larvae were fed with fish food (Tokyu®) and reared to 4^{th} instars which were used in the experiments.

Tubificid larvae were collected from sewage drain of North Kolkata, India. They were separated from clumped population and maintained in an enamel tray under slow running tap water in laboratory between 25 to 30 °C temperature.

Vegetables (Spinach) from local market were cut into pieces so that guppy could consume them easily.

Experiment under two different habitat conditions

Predatory preference of guppy was studied in a three-prey system under different social and physical conditions. These included foraging alone or alongside male conspecifics in simple or complex habitat. The study consisted of two related but separate experiments.

In case of simple habitat, trials took place in aquaria (15×15×15 cm) containing 2.5 lit. of tap water. In case of complex habitat the laboratory microcosms were constructed in glass aquaria (size 15×15×15 cm) using pebbles and aquatic weeds (duck weed and *Hydrilla*) and water (tap water + pond water in 1:1 ratio, total water 2.5 lit). Guppies were introduced to experimental aquaria at least 24 hours (overnight), before a trial in order to settle.

Now in glass aquaria of both simple and complex habitat 150 prey of three types - 50 *Aedes* larvae, 50 tubificid larvae and 50 pieces of vegetables were provided to a *Poecilia reticulata* (P1) to observe its predatory preference. After 1 hr. of feeding, the fish was removed and the numbers of prey alive were counted. This value was subtracted from the number of prey provided to obtain the number of prey consumed by the fish. Six replicates were done for this experiment. The experiment was repeated using the predator densities of two males (P2) and four males (P4) with six replicates for each.

Manly-Chesson index (Chesson, 1978; 1983) was calculated to evaluate prey selection. The index formula is:

$\alpha = (r_i / p_i) / \Sigma (r_i / p_i)$, $i = 1, 2, ..., m$,

with r_i = the proportion of food item *i* in the diet and p_i= the proportion of food item *i* in the environment and m = the number of food items in the environment. If $\alpha = (1/m)$, it means that a prey is consumed in proportion to abundance in environment, whereas $\alpha > (1/m)$ indicates preference and $\alpha < (1/m)$ indicates avoidance.

Niche breadth (B) for the fish was determined using the following formula (Levins, 1968)

$B = -\Sigma^{n}_{i=1} r_i * \log r_i$

The niche breadth can be used as an indicator of adaptability of *Poecilia reticulata* as a general predator.

Statistical analysis

The data on prey consumption were subjected to statistical analysis to justify the effects of complex habitat and prey types (tubificid and vegetables) on the selection of *Aedes* larvae by *Poecilia reticulata* and the effects of density on the rate of consumption of the three prey species.

The effects of density on the rate of consumption were evaluated through one-way factorial ANOVA. Also, a paired data t test was carried out to justify the variation in feeding and niche breadth between the habitat types. The preference of the mosquitoes was determined using a *t*-statistic for deviation from 0.33 (value > 0.33 indicate a relative preference while value < 0.33 indicate a relative avoidance). For the food types, a value less than or more than expected were subjected to a *t*-test to justify significant relative avoidance and relative preference. The data were analyzed in Excel 2003 (Microsoft Seattle, WA, USA) with the add-in software Statcel 2 (Yanai, 2004).

RESULT

The number of total prey consumed by *Poecilia reticulata* varied significantly (t_{17}= 3.37; $p < 0.01$) with different habitat conditions (simple habitat- 67.88 ± 2.62 and complex habitat- 76.33 ±3.53), being high in complex habitat (Table 1).

In the presence of alternative prey forms (tubificid worms and vegetables) the vulnerability of the mosquito immature lowered to a greater degree in case of simple habitat and the vulnerability of the mosquito immature increased to a greater degree in case of complex habitat, reflected through the Manly-Chesson index (Chesson, 1978 and 1983) (Table 2).

Student t test showed relative numbers of different prey consumed varied significantly between habitat conditions, being high for mosquito larvae and vegetables in complex habitat and tubificid for simple habitat (Figure 1).

Single factor Anova revealed that fish foraging differed significantly between predator densities both in simple (($F_{2,15}$=12.88, cd=11.65, ANOVA P<0.05 between $T_{P1} – T_{P2}$ and $T_{P1} – T_{P4}$, $F_{2,15}$=173.07, cd= 11.65, ANOVA P<0.05 between $ML_{P1} – ML_{P2}$, $ML_{P2} – ML_{P3}$ and $ML_{P1} – ML_{P4}$ and $F_{2,15}$=5.90, cd=25.63, ANOVA P<0.05 between $V_{P1} – V_{P2}$ and $V_{P2} – V_{P4}$: T= Tubificid larvae, ML=Mosquito larvae, V= Vegetables and cd=critical difference) and complex habitat ($F_{2,15}$=39.42, cd=27.37, ANOVA P<0.05 between $T_{P1} – T_{P2}$, $T_{P2} – T_{P3}$ and $T_{P1} – T_{P4}$, $F_{2,15}$=4.27, cd= 40.12, ANOVA P<0.05 between $ML_{P1} – ML_{P4}$ and $F_{2,15}$=4.29, cd=15.71, ANOVA P<0.05 between $V_{P1} – V_{P2}$ and $V_{P2} – V_{P4}$) but the trend of selection remained same (Figure 2a & Figure 2b).

Thus, there was significant interaction between the effects of habitat and social factors.

Table 1. Total prey consumption vs. habitat

Habitat	Total No. of Prey Consumed
Simple habitat	67.88 ± 2.62
Complex habitat	76.33 ±3.53

Significant difference (t_{17}= 3.37; $p < 0.01$)

Table 2. The selectivity index and niche breadth vs habitat

Habitat Type	Selectivity Index of Prey Types	Niche breadth
Simple habitat	Tubificid: 0.683±0.05	0.297±0.009
	Aedes Larvae: 0.203±0.05	
	Vegetables: 0.1030±.029059	(0.2-0.34)
	Tubificid worms (selectivity coefficients >0.33, $t_{(5)}$= 8.08; $p < 0.001$); there was a mean preference parameter value for Mosquito larvae of below <0.33 in all cases	
Complex habitat	Tubificid: 0.31± 0.045826	0.351±0.007
	Aedes Larvae: 0.516±0.026	
	Vegetables: 0.156±0.020	(0.3-0.39)
	(selectivity coefficients >0.33, $t_{(5)}$= 6.74; $p < 0.01$); there was a mean preference parameter value for Mosquito larvae of >0.33 in all cases	The niche breadth between simple and complex habitat vary significantly ($t_{(17)}$= 5.92; $p < 0.001$) .

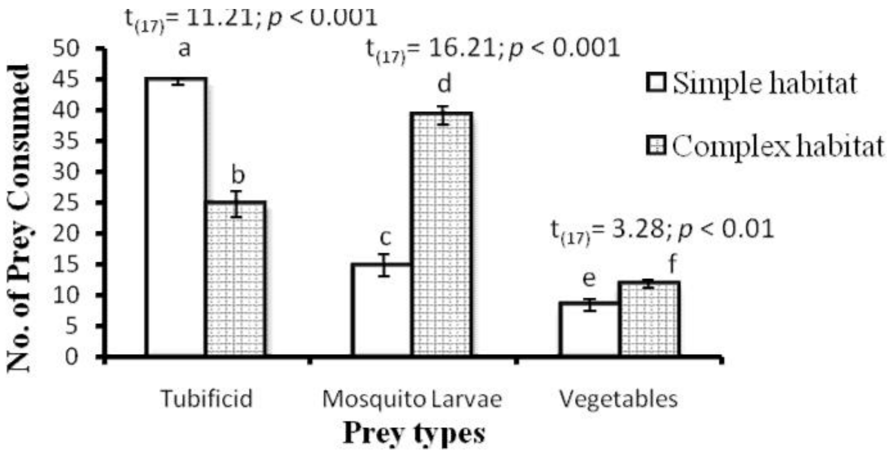

Figure 1. The relative numbers of different prey consumed at different habitat

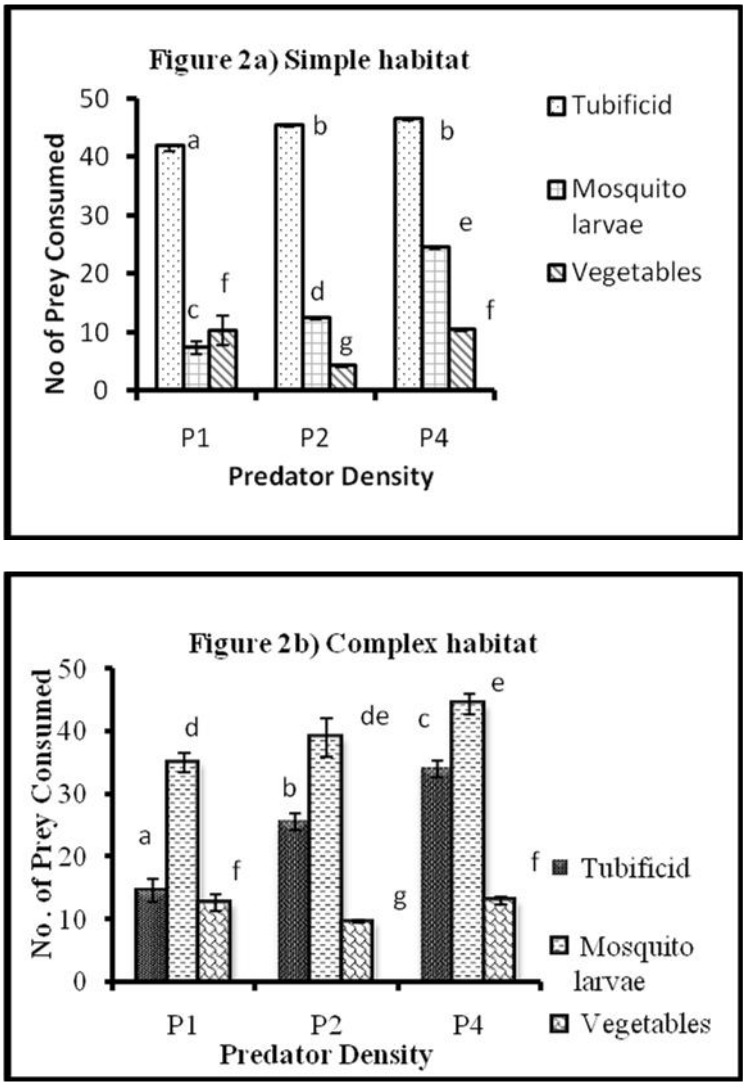

Figure 2. Relative consumption rate (mean ± S.E.) of different prey types at three different predator densities of *Poecilia reticulata* (n= 6 replicates per predator density)

DISCUSSION

This study revealed convincing evidence for the occurrence of prey preference in the guppy and, is affected by several variables. A general predator utilising a variety of prey resources can adapt and thrive well in a wide range of habitats. The exotic fish *Poecilia reticulata* is such an example, which has a wide range of dietary choices. Added to this is their ability to switch between abundant prey forms (Warburton and Thomson, 2006). These favour the use of the *Poecilia reticulata* in biological control of mosquitoes.

In simple tap water condition, mosquito larvae remained in aggregate on the water surface and tubificid worms on the bottom of the aquaria. In course of experiment, it was observed that *Poecilia reticulata* oriented for the prey mostly on the water surface with irregular exploration in the mid column of the aquarium (Aditya *et al*., 2012). In spite of such spatial distribution of prey and predators guppies consistently preferred tubificid when given a choice between tubificid, vegetables and *Aedes* larvae at simple habitat. Similar findings were reported by Aditya *et al*., 2012. Tubificid worms were caught in groups whereas mosquito larvae were captured individually by fish significantly lower selectivity value for mosquito larvae. The mouth gape of the larvivorous fish possibly contributed to the difference in the prey selection pattern. In case of complex habitat the effective space for movement was restricted due to presence of pebbles and aquatic weeds and the fishes remained for more time in the mid-column of the space. In complex habitat, *Aedes* larvae present in the mid column and surface of aquarium so guppy consumed more mosquito larvae. In the complex habitat guppy could not find out tubificid worms easily as tubificid used the pebbles as refuge and oriented in the spaces between the pebbles. Several studies have found that increased habitat heterogeneity, and the presence of refugia, can reduce predatory impact in aquatic communities (Diehl, 1992; Bechara *et al*., 1993).

The prey types IVth instar *Aedes* larvae and tubificid worms showed clumped orientation in space. The clumping pattern of mosquito larvae in complex habitat differed from simple habitat conditions, few single individuals oriented in the open spaces. Movement of individuals from one patch to another was noticed with most frequent for the mosquito larvae and least for the tubificid worms. Less active prey types might gain a better advantage from habitat structure than more active species which highlight the importance of behavioural differences between alternative prey species to the effect of habitat complexity. Baber and Babbitt (2004) investigated the effect of habitat structure on *Gambusia holbrooki* prey preference and found similar result. Relationship between habitat complexity and predation efficacy is not always straight forward. It depends critically on how the habitat is used by both predators and prey (Savino and Stein, 1989). Presence of alternative prey species reduced the efficacy of predators (Koss and Snyder, 2005; Symondson *et al*., 2006).

From the results it is apparent that *Poecilia reticulata* fish consumed *Aedes* mosquitoes at higher numbers in the complex habitat conditions, in presence of alternative prey types. These were consumed at a higher rate than simple habitat conditions. Thus, the foraging pattern of *Poecilia reticulata* differed between the habitat conditions. The reason for variations of niche breadth could be due to the resource selectivity by the individual from the environment (Petraitis, 1979). Overall, there was no significant difference in the total number of prey items consumed per trial between fish experiencing the trials alone, or with two and four male conspecifics. The relationship between consumption rate of prey and predator densities indicate that the dengue vectors were the preferred prey of male guppies in the complex system and tubificid in simple habitat.

The effective vectors of Dengue are semi domestic and domestic mosquitoes (Chan *et al*., 1971; WHO, 1997). Water storage containers, discarded containers, tyres and other vessels, water collection wells of closed underground drains, garden stone pools, blocked cement drains and septic tanks are the key breeding sites of *Aedes* (Chang *et al*., 2011). Among them, large indoor household water containers that contain clean water are the most frequent breeding sites for *Aedes* (Neng *et al*., 1987; Ghosh *et al*., 2011). *Aedes* normally do not breed in wet lands till some samples could be available (Haider *et al*., 2013). Selectivity for *Aedes* larvae in complex habitat in presence of alternative prey and male conspecifics suggests that male guppy may be effective in regulating mosquitoes in wetlands. The foraging behaviour of male and female guppies can be quite distinct with females tending to consume greater numbers of prey (Elias *et al*., 1995). Simultaneously sexual harassment by male guppies weakens preference for the preferred prey of females (Croft *et al*., 2006; Ojanguren and Magurran, 2007; Darden and Croft, 2008). The present research suggests ineffectiveness of female guppies in natural wetland (complex system) as biocontrol agent might be due to their feeding at a non preferred prey patch to minimise male harassment. Conversely, as the vector species was not the preferred prey, male harassment could increase effectiveness by forcing females to feed more upon dengue vector population in simple habitat of household water containers. Similar bio control with Chinese cat fish significantly reduced the house and container indices of the larval population were reported by Neng (1987) and Phuanukoonnon *et al*. (2005). Integrated control of *Aedes* was done in a coastal village of Taiwan using *G. affinis*, *P. reticulata*, *Tilapia mossambica* and *Sarotherodon niloticus* in potable water containers (Wang *et al*., 2000). In Southern Mexico *P. reticulata* were significantly effective as biocontrol agents against *Ae. aegypti* larvae in water storage tanks (MartiNez-Ibarra *et al*., 2002). More recently, in the northeastern Brazilian state of Ceará *P. Reticulata* was successfully used as a non-native larvivorous fish species to combat *Ae. aegypti* larval infestation (Pamplona, 2006). In Cambodian villages *P. reticulata* reduced dengue-carrying *Ae. Aegypti* larval infestation by 79%, compared to control villages (Seng *et al*., 2008). Predatory nature of this fish may also inhibit *Aedes* oviposition in domestic containers (Pamplona *et al*., 2009). *Poecilia* survives better in confined habitats as well as in small containers with minimum care (Ghosh *et al*., 2011). In absence of mosquito larvae, this fish can survive on alternative feed available within the ecosystem (Ghosh *et al*., 2011).

CONCLUSION

The present foraging study suggests that guppies are indeed capable of effectively regulating *Aedes* populations, and this would indicate that, at least in some circumstances, their introduction may be justified.

The bioecology of guppy indicates that they are found to feed on a wide range of plankton and insect species including mosquito. Therefore, it needs to be explored further whether the predation of *Poecilia reticulata* is affected by the presence of alternative prey. Further, information regarding the positive and negative impacts of guppies on native species and ecosystems, when introduced, is extremely scarce. Cost benefit analyses have been recommended to justify any possible risk to native fauna in terms of positive impacts on control of dengue epidemics. In this context assessment of the efficacy of the indigenous larvivorous fish species under varying habitat conditions using both male and female should be carried out prior to promoting the exotic fish *Poecilia reticulata* in dengue control programme.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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