

## Research Articles

# Vulnerability of the mosquito larvae to the guppies (*Poecilia reticulata*) in the presence of alternative preys

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## Abstract

**Background & objectives:** The predatory potential of the larvivorous fishes can be affected by the presence of alternative preys. In the present study the predation pattern of the sewage dwelling *Poecilia reticulata* (Peters 1872) on the larvae of *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) was evaluated in the presence of alternative preys.

**Methods:** The predation of *Cx. quinquefasciatus* larvae by different size groups of *P. reticulata* fishes was evaluated. In addition to this, the niche breadth (*N*) and diet breadth (*B*) were measured following Manly's selectivity index (*S<sub>i</sub>*) as an indicator of variation of such predation pattern in the presence of alternative prey types, like chironomid larvae and tubificid worms.

**Results:** The consumption of IV instar *Cx. quinquefasciatus* larvae by individual *P. reticulata* ranged between 65 and 84 in a 3 h feeding period and varied with the size of fish ( $F_{2,33} = 34.91$ ;  $p < 0.001$ ). The selectivity coefficient revealed a significantly low preference for the *Cx. quinquefasciatus* larvae (0.16, CL: 0.05 – 0.27;  $p < 0.05$ ) compared to the chironomid larvae and tubificid worms, when all the three prey types were present. The niche breadth (*N*) and diet breadth (*B*) ranged from 0.77 to 0.92 and 0.69 to 0.93, respectively. The total consumption of all the prey types varied with the predator density, but the selectivity index for the mosquito larvae was significantly low in all the instances.

**Interpretation & conclusion:** *P. reticulata* can consume a good number of mosquito larvae, with the consumption rate varying with the body size. *P. reticulata* fishes exhibit low preference for mosquito larvae as prey in the presence of alternative controphic preys like chironomid larvae and tubificid worms. Though establishment and sustenance of *P. reticulata* in new habitats will be favoured by the presence of alternative preys, but vulnerability of mosquito larvae may be reduced with availability of multiple preys in natural conditions.

**Key words** Chironomids – *Culex quinquefasciatus* – guppies – larvae – *Poecilia reticulata* – predation – sewage drain – tubificid worms

## Introduction

The larvivorous fish, *Poecilia reticulata* is an effective biological control agent of mosquitoes in differ-

ent habitats in India, particularly in Kolkata, India<sup>1-4</sup>. These fishes have adapted to the sewage drain conditions and are involved in regulating the populations of different species of pest and vector mosquitoes

including *Culex quinquefasciatus* (Diptera: Culicidae)<sup>1</sup>. The regulation of the mosquito immatures by the natural predators are affected by the presence of alternative prey forms as has been noted in case of the dytiscid beetles<sup>5</sup>, cyclopoid copepods<sup>6</sup>, odonate nymphs<sup>7,8</sup> and the mosquito fishes<sup>9–12</sup>. Apart from the mosquito immatures, the sewage drains are natural habitats for the chironomid immatures and tubificid worms. Thus, the presence of these alternative prey species can affect the predation pattern and thus the biocontrol efficiency of the guppies. In view of these, the predatory efficiency and prey selection of the sewage drain inhabiting guppies was assessed using the larvae of *Cx. quinquefasciatus* as preys in the presence of alternative prey types. The alternative prey types considered were the controphic species — chironomid larvae and tubificid worms. The results of the present study are expected to highlight the selection pattern of mosquito larvae by these fishes in the presence of alternative prey type. Further, the role of these fishes in structuring the sewage drain communities can be assessed.

### Material & Methods

Guppies were collected from the sewage drains near Garia, Kolkata, India and were kept in the laboratory in batches of four in glass aquaria of 15×15×12" containing 25 L of water (pond: sewage :: 1:1), at a temperature of 27–30°C. The fishes were maintained with Tokyu® fish food for a week prior to the experiments. Three size groups were selected—2.5, 3 and 3.5 cm corresponding to the mean length of the snout to the caudal tip of the individual fishes. Chironomid larvae and tubificid worms were collected through dredging the mud bottoms of the same sewage drain. The collected preys were placed in separate enamel trays under flowing tap water in the laboratory. The tubificid worms were mostly *Tubifex* and *Branchiodrillus* sp, while the chironomid immatures were mostly of the genus *Chironomus* sp. The tubificid worms of 15–20 mm in length and the chironomids of 15–20 mm (corresponding to late IV instar

larvae) were separated from the population and used as preys. The larvae of *Cx. quinquefasciatus* were obtained from a sewage drain. The heterogeneous larval population collected from the drains was sieved in the laboratory in a mixture of sewage drain and tap water initially to separate out the IV instar (5.1–6 mm) stages. The smaller instars thus obtained were further sieved to obtain the II instar (1.5–2.5 mm). The rest of the larval population was reared in enamel trays with Tokyu® fish food *ad libitum*. The prey animals were maintained in the laboratory under optimal conditions of temperature 25–30°C, pH 8.9–9.7. The predators were offered ample amount of food to meet their satiation level and then starved for 24 h before utilizing them for the experiments. In all the experiments, controls without predators were set with equal number of replicates as that of the test.

In the first experiment, guppies of the three size groups were exposed to a feeding bout of three hours with 100 IV instar larvae of *Cx. quinquefasciatus*. The rate of feeding was recorded at an interval of 10, 20, 30, 60 and 180 min. Twelve replicates of each size group of the fish predators were considered. The data obtained were subjected to one-way ANOVA to justify the difference in the feeding rate between the size groups. Also, a two-way factorial ANOVA was carried out to justify the variation in feeding between the time intervals.

In the second round of experiments, 300 preys of three types—IV instar *Cx. quinquefasciatus*, chironomid larvae and tubificid worms were provided in equal proportions to the guppies of a particular size group (3.5 ± 0.11 cm body length) and the data on the rate of predation were noted after 3 h. The experiment was repeated using the predator densities of 1, 2 and 4 predators with six replicates for each. The effects of density on the rate of consumption of the three prey species were evaluated through two-way factorial ANOVA.

The data obtained on predation were further sub-

jected to the analysis of selectivity, niche breadth and diet breadth of the predators. The following formulae were applied following Rehage *et al*<sup>12</sup> and Krebs<sup>13</sup> for such analysis.

$$w_i = c_i/a_i$$

$$S_i = w_i/\sum w_i$$

Where,  $w_i$  = Prey preference,  $c_i$  = Proportion of the preys consumed,  $a_i$  = Proportion of the prey available;  $S_i$  = Equivalent to Manly's  $\alpha$  (selectivity index).

The preference of the mosquitoes was determined using a *t*-statistic for deviation from 0.33. Since three prey species were available, any value > 0.33 will indicate a relative preference for that particular prey species while any value < 0.33 will indicate a relative avoidance for that prey type. For mosquito larvae, a value of <0.33 and for chironomid larvae and tubificid worms a value of >0.33 were tested following Zar<sup>14</sup> to justify relative avoidance (of mosquito larvae) and relative preference (of alternative preys), respectively.

The niche breadth  $N$  and the diet breadth  $B$  were determined by:

$$N = 1/\sum(c_i^2/a_i)$$

and

$$B = (N - a_{\min})/(1 - a_{\min}); \text{ which in the present case equals to } (N - a_i)/(1 - a_i)$$

Here,  $a_{\min}$  represents the smallest proportion of the prey available ( $\approx 100/300$  or  $a_i$ ). Since the preys were provided in equal proportions, therefore, the value is equal to  $a_i$ . The niche breadth and diet breadth can be used as an indicator of adaptability of *P. reticulata* as a general predator. The values are representations of the pattern of utilization when multiple resources are available.

## Results

The number of IV instar *Cx. quinquefasciatus* larvae consumed by *P. reticulata* varied with the size (Fig. 1), which can be represented as—Consumption  $y = 21.26 + 17.5x$  body length ( $F = 71.93$ ;  $p < 0.001$ ;

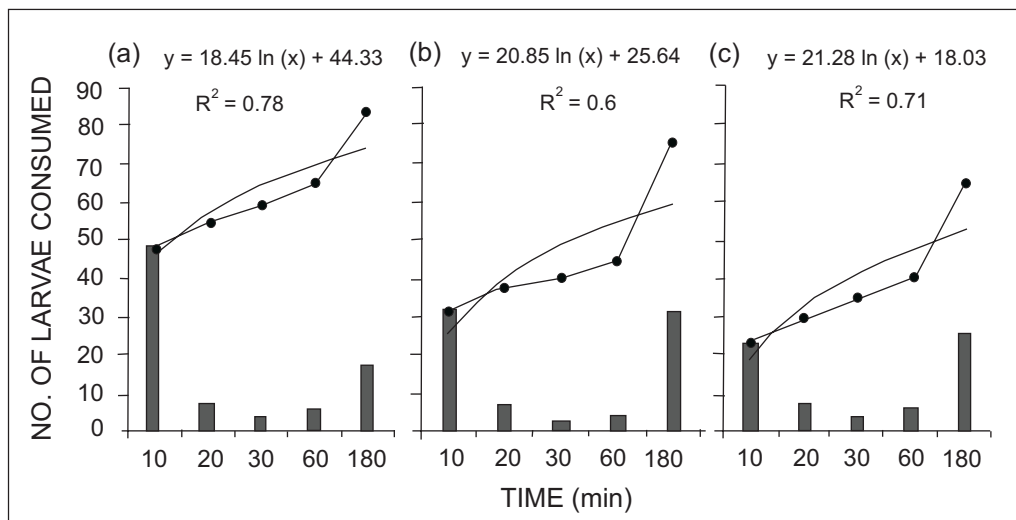


Fig. 1: The predation pattern of *P. reticulata* of three different size groups [(a) 2.5 cm, (b) 3.0 cm, and (c) 3.5 cm fish size] on the IV instar mosquito larvae in a three-hour feeding bout ( $n = 12$  per predator size group). Bar represents mean consumption at that interval of time and the line represents the mean cumulative consumption at that time interval

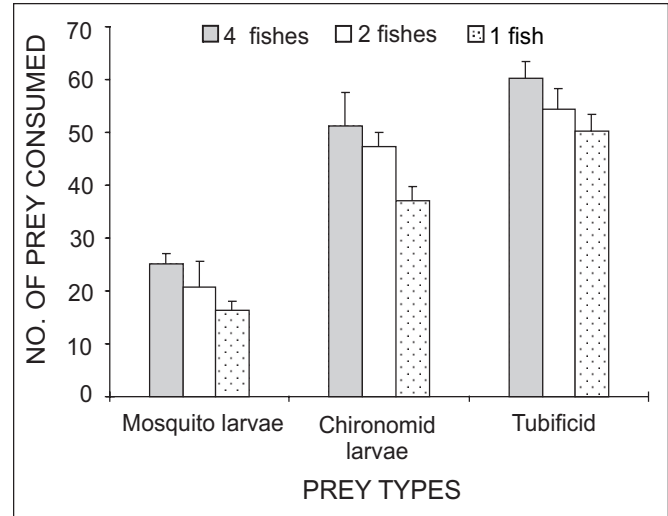
**Table 1. Results of two-way factorial ANOVA on the effects of size group and time interval of feeding in *P. reticulata* (n = 12 observation per size group)**

Source of variation	Sum of squares	df	Mean square	F
Time interval	25765.58	4	6441	179.8
Size of guppy	444.68	2	222.3	6.205
Time* Size of guppy	4001.88	8	500.2	13.96
Error	5911.92	165	35.83	
Total	36124.06	179		

All F-values significant at  $p < 0.001$  level.

$R^2 = 0.679$ ). In the total 3 h feeding bout, the mean number of prey consumed ranged between 65 and 84 depending on the body length of the predators ( $F_{(2,33)} = 34.91$ ;  $p < 0.001$ ). Further, the two-way factorial ANOVA revealed that the prey consumption of the guppies varied between the time intervals within the 3 h period as well as between the body sizes, significantly (Table 1), indicating the searching and handling is interrupted by the utilization of the prey by the physiological system of the predators.

The consumption rate of the guppies varied with the predator densities but the trend of prey selection remained same (Fig. 2). Two-way factorial ANOVA revealed that total prey consumption differed significantly between the predator densities as well as between prey types (Table 2). The post-hoc test (Tukey test) revealed significant differences ( $p < 0.001$ ) in relative consumption of all the three prey types (between mosquito and chironomid larvae,  $|q| = 24.5 \pm 3.1$ ; between mosquito and tubificid,  $|q| = 34.22 \pm 3.1$ ; between tubificid and chironomid larvae  $|q| = 9.72 \pm 3.1$ ), irrespective of the predator densities ( $p < 0.001$ ). In the presence of alternative prey forms (chironomid larvae and the tubificid worms) the vulnerability of the mosquito immatures lowered to a greater degree, reflected through the Manly's selectivity index (Table 3). The *t*-test showed that the selectivity coefficients for IV instar *Cx. quinquefasciatus* larvae

**Fig. 2:** Relative consumption rate (mean  $\pm$  S.E.) of three different prey types at three different predator densities of *P. reticulata* (n = 6 replicates per predator density)

were  $< 0.33$  significantly, at all the predator densities. In contrast to this, the selectivity coefficients were  $> 0.33$  significantly, for the tubificid worms. For the chironomid larvae as prey, the selectivity coefficients were  $> 0.33$ , but not significant. This indicates that the predator selected the alternative prey more than the mosquito larvae, when all the prey types were present at equal densities. The niche breadth and the diet breadth did not vary much with the predator densities, reflecting that the increase in density did not

**Table 2. Results of two-way factorial ANOVA on the effects of predator density and prey types on the consumption pattern of *P. reticulata***

Source of variation	Sum of squares	df	Mean square	F
Predator density (D)	1095.26	2	547.63	6.34*
Prey type (PT)	11195.59	2	5597.80	64.80*
D* PT	88.74	4	22.19	0.26 NS
Error	3887.67	45	86.39	
Total	16267.26	53		

Level of significance \* $p < 0.01$ ; \* $p < 0.001$ ; NS = Not significant.

**Table 3. The selectivity index, niche breadth and diet breadth of *P. reticulata* adapted to sewage drains**

Parameters		No. of predators		
		1	2	4
<i>Selectivity index (Si)</i>				
<i>Cx. quinquefasciatus</i> larvae	LCL–UCL	0.05–0.27	0.03–0.32	0.14–0.25
	<i>Si</i> ± S.E.	0.16 ± 0.04	0.18 ± 0.06	0.2 ± 0.03
	<i>t</i> -value	3.85*	2.67*	3.5*
Chironomid larvae	LCL–UCL	0.18–0.56	0.17–0.60	0.04–0.64
	<i>Si</i> ± S.E.	0.36 ± 0.07	0.39 ± 0.08	0.34 ± 0.12
	<i>t</i> -value	0.55 NS	0.69 NS	0.11 NS
Tubificid worms	LCL–UCL	0.34–0.63	0.33–0.53	0.24–0.68
	<i>Si</i> ± S.E.	0.48 ± 0.06	0.43 ± 0.04	0.46 ± 0.09
	<i>t</i> -value	2.67*	2.74*	1.5 NS
<i>Niche breadth (N)</i>		0.79–0.92	0.79–0.92	0.77–0.91
		0.84 ± 0.05	0.87 ± 0.05	0.85 ± 0.05
<i>Diet breadth (B)</i>		0.68–0.89	0.68–0.93	0.66–0.87
		0.77 ± 0.07	0.83 ± 0.06	0.77 ± 0.07

LCL – Lower confidence limit; UCL – Upper confidence limit; \*Significant at  $p < 0.05$  level; NS–Not significant.

shift the prey consumption as well as selection pattern of *P. reticulata*.

### Discussion

*P. reticulata* fishes are efficient in regulating the mosquito larvae, as are the other fishes, like *Gambusia affinis*<sup>1–4</sup>. The prey consumption ability of these larvivorous fishes increases with the body size. In the present study, this is reflected in the predation of IV instar *Cx. quinquefasciatus* larvae by the *P. reticulata* adapted to the sewage drains. From the view of biological control, however, the regulation of the mosquito population depends on the prey selection pattern by the predators. A general predator utilising a variety of prey resources can adapt and thrive well in a wide range of habitats. The exotic fish *P. reticulata* is such an example, which has a wide range of dietary choices and thus have adapted well in the sewage drains. Added to this is their ability to switch between abundant prey forms<sup>15</sup>. These favour the use of the *P.*

*reticulata* in biological control of mosquitoes.

The sewage drains and several other mosquito larval habitats where these fishes have adapted well as predator, other alternative prey forms are also available. The prey preferences of *P. reticulata* in such situations need to be inclined more towards the mosquito immatures. In the context of the observations made in the present study, the fishes fail to choose more of the target prey—the mosquito larvae. Alternative preys allow sustenance of the predators in the absence of the target prey species. But, in instances when the preference of the predators for the alternative prey forms is high compared to the co-occurring target prey, the regulation of the target prey population may be reduced. In other cases, the predators like some dytiscid beetles<sup>5</sup> and the copepods<sup>6</sup> may show a positive selectivity of mosquito larvae against other prey forms. Evidences in cases of some dytiscid beetles<sup>5</sup>, and odonate nymphs<sup>7,8</sup>, and mosquito fishes<sup>9–11</sup> have shown that alternative prey forms af-

fects the predation of target prey. In rice-fields, as indirect effects, the presence of mosquito fish, *G. affinis* brought about the faster growth of the mosquito, *Cx. tarsalis* in the presence of the cladocerans<sup>9,10</sup>. In another observation, the mosquito fish depressed the population of hemipteran aquatic bugs but could not reduce the population of the mosquitoes at significantly lower levels<sup>11</sup>. Therefore, it seems that under natural conditions, presence of alternative prey reduces the possibility of local extinction of the mosquito populations. Besides, the indirect effect on the community members can alter the community structure and function. For the guppies in the sewage drains, such possibilities can not be ruled out, though field observations on the population cycles of the fishes and the mosquitoes are required to evaluate this. Since marked variations in population structure including morphological, physiological traits and the predatory ability of the guppies can have a bearing on the population regulation of mosquito immatures<sup>16</sup>.

The preference pattern exhibited by *P. reticulata* supports that in case of augmentative release in sewage drains or similar habitats, non-target preys would have a positive effect on the establishment of the fishes in those habitats. As a consequence this would have a negative impact on the colonization of the mosquitoes apart from being predated, if present in the habitats. However, the vulnerability of the mosquito larvae to the guppies may be affected if other controphic preys are present in such habitats. Thus, the direct and indirect effects of the presence of guppies in the mosquito larval habitats can be expected. The results of the present study support such view and call for strict surveillance of the guppies in the sewage drains of Kolkata and elsewhere. *P. reticulata* fishes are definitely required as a part of the biological control programme, but field evaluation on the details of their predation ecology is required<sup>11,12,15</sup> to assure proper functioning as biocontrol agents, particularly in the tropical and subtropical countries like India, where the rich biodiversity might be affected.

## Acknowledgement

The authors are grateful to the respective Heads, Department of Zoology, University of Calcutta, Kolkata and the University of Burdwan, Burdwan, for the facilities provided. BM thankfully acknowledges the financial support from the University of Calcutta, Kolkata in carrying out this work.

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*Received:* 31 January 2008

*Accepted in revised form:* 27 April 2008