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Efficacy of indigenous larvivorous fishes against *Culex quinquefasciatus* in the presence of alternative prey: Implications for biological control

Gautam Aditya[1,2], Santanu Pal[1], Nabaneeta Saha[2,3] & Goutam K. Saha[2]

[1]*Ecology Laboratory, Department of Zoology, The University of Burdwan, Burdwan;* [2]*Department of Zoology, University of Calcutta, Kolkata;* [3]*Serampore College, Serampore, Hooghly, India*

ABSTRACT

Background & objectives: Indigenous larvivorous fishes bear potential for regulating vector mosquitoes through trophic interactions. The mosquito prey preference of five indigenous larvivorous fishes in the presence of alternative food items was assessed to highlight their use in mosquito vector management.

Methods: Laboratory experiments were carried out using the larvivorous fishes *Ambassis (=Chanda) nama*, *Parambassis (=Chanda) ranga, Colisa fasciatus*, *Esomus danricus* and *Aplocheilus panchax*, as predators and IV instar *Culex quinquefasciatus* larvae as target prey. Mosquito prey preference of these fishes in the presence of chironomid larvae, tubificid worms and artificial fish foods, in varied proportions, were assessed using preference index.

Results: The fishes consumed considerable amount of mosquito larvae both in absence and presence of alternative food items. However, the positive selectivity for mosquito larvae at all densities were significantly (p <0.05) affected by the alternative foods. The chironomid larvae and tubificid worms were consumed proportionately higher than expected (p <0.05), while the artificial fish food was consumed at lower than expected proportions (p <0.05). The niche overlap was significantly similar among the fish species suggesting likeliness in predation pattern and prey preference.

Interpretation & conclusion: The results reflect that the alternative food items influence the mosquito prey selectivity and thus the efficacy of indigenous larvivorous fishes. While use of these fishes in the wetlands and allied mosquito larval habitats in different parts of the country is suggested, impact of the alternative prey may affect the successful regulation of mosquitoes. Assessment of appropriate predator-prey ratio under natural habitat conditions is recommended to enhance successful mosquito control by these fishes.

Key words Biological control; chironomid larvae; indigenous larvivorous fish; mosquito larvae; prey preference; tubificid worms

INTRODUCTION

Biological control of mosquitoes employing larvivorous fishes can be a sustainable practice[1]. The guppy *Poecilia reticulata* Peters and mosquito fish *Gambusia affinis* (Baird and Girard) have long been promoted for biological control of mosquitoes and their success has been documented on many instances[1–4]. However, considering their invasive nature and failure to provide desired results in certain instances[5–7], the indigenous larvivorous fishes are being promoted as an alternative option[8–11]. Vector mosquito regulation is aptly demonstrated by using indigenous larvivorous fishes in Argentina[8] and Mexico[9]. In India, several indigenous fishes possess the potential to regulate mosquito populations[10, 11]. Empirical evidence is provided from the studies of *Macropodus cupanus* Valenciennes[12, 13], *Aplocheilus lineatus* Valenciennes (Cyprinidontiformes: Aplocheilidae)[14], *Colisa fasciatus* Bloch and Schneider (Perciformes: Osphronemidae), *Aplocheilus panchax* Hamilton-Buchanan (Cyprinidontiformes: Aplocheilidae), *Rasbora daniconius* Mukerji (Cypriniformes: Cyprinidae), *Ambassis nama* Hamilton (Perciformes: Ambassidae) and *Esomus danricus* Hamilton (Cypriniformes: Cyprinidae)[15–17]. Several of these indigenous larvivorous fishes have been recorded from ricefields and temporary pools[10,11]. This provides a fair possibility of their use in regulating mosquitoes in wetlands.

In view of the potential for mosquito control by native larvivorous fishes, the present study was aimed at evaluating the prey preference of five indigenous species, using *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) as target prey. The fish species considered are *Ambassis (=Chanda) nama*, *Parambassis (=Chanda) ranga, Colisa fasciatus*, *Aplocheilus panchax* and *Esomus danricus*. A comparison with the exotic fish *P. reticulata* was also made. The results of the present study will help to evaluate the efficacy of these indigenous fishes in regulating vector mosquitoes, apart from promoting the significance of conservation of these indigenous species. The indigenous larvivorous fishes are general in their dietary choice, so prey preference is important when evaluating their potential as biological control agents against mosquitoes. 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[5–7, 18]. Thus, assessment of mosquito preference is a pre-requisite to promote these fish species for biological control. Mosquito prey selectivity of indigenous fish species will facilitate selection of appropriate species in regulation of wetland mosquitoes as a part of conservation biological control. The possible impact of alternative prey in impeding mosquito control by the indigenous fishes can also be predicted from the results of this study.

MATERIAL & METHODS

Collection and maintenance of fishes

The five fish species *Ambassis (=Chanda) nama*, *Parambassis (=Chanda) ranga, Colisa fasciatus*, *Aplocheilus panchax*, and *Esomus danricus* were collected from the rice-fields and irrigation canals in and around The University of Burdwan campus and Agricultural Farm, Golapbag, Burdwan, India[19]. The fish specimens with standard total length ranging between 33 and 45 mm were brought to the laboratory, segregated, and maintained separately species-wise in earthen tubs of 60 L capacity (at a density of 25 fishes/60 L). Few specimens of the macrophytes *Lemna* sp, *Pistia stratiotes* and *Alternenthera philoxeroides* collected from the irrigation canals were added to the tubs to simulate natural conditions. The fishes were provided in equal ratio of tubificid worms, chironomid and mosquito larvae *ad libitum* as food and 30 L of water was changed every other day. The exotic fish, *P. reticulata* ranged between 27 and 35 mm in standard length was collected from sewage drains near The University of Burdwan campus, Golapbag, Burdwan, within the limits of Burdwan Municipality area. The fish were maintained in a similar way mentioned above. Fish were acclimatized for at least seven days in the laboratory. Prior to the experiments all the individuals were fed to satiation (till excess of food was noticed in the tubs) and then starved for 24 h.

The tubs contained aerated tap water and were preferably placed in an open area, under natural light with temperature ranging between 23 and 32°C and pH between 7.7 and 8.1. Biological filtration in the tubs was obtained through pebbles and sediment. For any experimental trial a fish was used only once. The collection of the fishes was continued throughout the experimental period (August to November 2008) and the newly collected fish individuals were replaced with equivalent number of older ones to keep the rearing density at 25 fish per 60 L.

Collection and maintenance of prey

The mosquito (*Cx. quinquefasciatus*) and chironomid larvae were collected from different sewage drains, bogs and cesspits in and around Burdwan Municipal area, adjacent to the University of Burdwan campus, following suitable methods[18, 20]. In the laboratory, the collected larvae were emptied in enamel trays to segregate larger one (IV instar) based on body length. The mosquito and chironomid larvae were maintained in transparent plastic trays separately. For chironomid larvae (~15–20 mm), the trays were under continuous aeration (SOBO No. SB-648A pump, China). Sewage sediment mixed with sand was present in the trays. In addition, crushed fish food (few grains of Tokyu®, Japan) was added as supplementary food. Commercial grade tubificid worms consisting primarily of *Tubifex* spp were procured from the local ornamental fish stores time to time as per requirements. In the laboratory, these were placed in an enamel tray with continuous water flow and aeration.

Experimental methods

Estimation of consumption of mosquito larvae: To assess predation potential and compare consumption rate of mosquito larvae, a single individual of each fish species was allowed to consume separately, 25, 50 and 100 IV instar *Cx. quinquefasciatus* larvae in a 14 L plastic bucket. The number of larvae consumed was noted at the end of two hour feeding bout[21]. The experiment was repeated using different fish individuals for each species. Twelve replicates were set per prey density for all fish species, and differences in prey consumption were analyzed using two way factorial ANOVA[22].

Estimation of preference for mosquito larvae: Prey preference for mosquito larvae by the fish species was evaluated using IV instar larvae of *Cx. quinquefasciatus* as target prey, chironomid larvae and tubificid as live preys and granular fish food (Tokyu® Corp, Japan) as alternative prey. A single fish was allowed to consume the foodstuffs supplied, either in equal proportion (50 each of the living specimens and 50 grains of fish food = 200 total) or low (25 mosquito larvae: 50 = 175 total) or high (50 mosquito larvae : 25 = 125 total) mosquito larvae in respect to other food types. The observations were made in a glass aquarium of 60 cm × 42 cm × 42 cm volume containing 80 L of tap water, for a period of two hours. The experiment was repeated 12 times for each of the predator fish species and prey proportions. For a particular fish species, three trials of a particular prey combination were carried out in a day using three different aquaria. In each trial, a different fish individual was used as a predator. The trials were made at different times since the number of a particular fish collected from the water bodies varied in a sample[19]. A fish individual in a trial represented randomly collected population from the water bodies and acclimatized in the tubs for seven days. The collection of the fish continued during the entire experimental period. This allowed interspersion and randomization such that the trials represent replicates. However, variations in the temperature conditions and the intensity of light were not controlled during the period of experiment coinciding with the rainy season. The data on predation were noted for each fish species and were subjected to preference analysis[21] and used elsewhere[18]. A preference for *i*th food item, *PPi* is obtained using the formula: $PP_i = PC_i / PA_i$; where, PC_i = Proportion of the *i*th food item consumed, PA_i = Proportion of the *i*th food item available. The selectivity index for a particular prey type is then calculated using an equivalent notation to Manly's α (selectivity index) (as proposed by Rehage *et al* [21], with changed notation): $MA_i = PP_i / \Sigma PP_i$. The niche breadth *NB* and the diet breadth *DB* were determined by $NB = 1/\Sigma(PC_i^2/ PA_i)$ and $DB = (NB–PA_{min})/(1–PA_{min})$, where, PA_{min} is the lowest proportion of food type available. The preference of the mosquitoes was determined using a deviation from expected 0.14 (for low mosquito relative density), 0.25 (for equal mosquito relative density) or 0.4 (for high mosquito relative density). Since four food types were available, any value above these will indicate a relative preference while any value below will indicate a relative avoidance for mosquito larvae. Corresponding to the proportion of mosquito larvae present, the preference for chironomid larvae, tubificid worms and artificial fish food was determined using a deviation from expected 0.29 (for low mosquito relative density), 0.25 (for equal mosquito relative density) and 0.2 (for high mosquito relative density). For the food types, a value less than or more than expected were subjected to *t*-test (one-tailed)[22] to justify significant relative avoidance and relative preference, respectively. A test of similarity of resource utilization by the fishes (niche overlap-based on Pianka's model[23]) was assessed using the software EcoSim[24]. The outline of the experiments is shown in Fig. 1.

RESULTS

Estimation of consumption of mosquito larvae

The native fishes consumed 10 to 33 IV instar *Cx. quinquefasciatus* larvae, depending upon the prey densities. In contrast, *P. reticulata* consumed 7 to 14 larvae (Fig. 1). The consumption rate differed among the fish species considerably, as evident from the results of two-way ANOVA (Table 1).

Preference for mosquito larvae by the larvivorous fishes

Predator fish consumed the four diverse food types but at varying numbers (Table 2). Native fish exhibited varying preference of mosquito larvae over other food items, respective of relative proportions available (Table 3). Apart from *A. panchax,* the fishes consumed mosquito larvae less than the expected values, even when the mosquitoes were present at higher proportion.

The artificial fish food grains were least consumed compared to the live prey. This may possibly be an adaptation of the indigenous fishes to predate upon live prey. In comparison, *P. reticulata* fed well on the fish food, a possible reflection of invasive nature and broad dietary choice. The niche breadth and the diet breadth varied with

Table 1. The results of two-way ANOVA and Tukey test on the consumption of IV instar larvae of *Cx. quinquefasciatus* by the larvivorous fishes. F-values in bold indicate significance at p <0.005 level

Source of variation	Sum of squares	df	Mean square	F
Fish species (FS)	1114.76	5	222.95	**11.818**
Prey density (PD)	4679.731	2	2339.866	**124.02**
FS * PD	490.88	10	49.088	**2.602**
Error	3735.5	198	26.121	
Total	10020.87	215		

Between fish species	Mean dif.	Significant (p <)
1 *vs* 6	5.1111	0.024
2 *vs* 6	5.1111	0.001
3 *vs* 4	3.6389	0.006
3 *vs* 5	3.0000	0.043
3 *vs* 6	3.4722	0.001
4 *vs* 6	3.8333	0.003
5 *vs* 6	4.4722	0.001

Fish species	Abbreviation	
	Numerical	Letters
Ambassis (=Chanda) nama	1	ANA
Aplocheilus panchax	2	APA
Colisa fasciatus	3	COF
Esomus danricus	4	EDA
Parambassis (=Chanda) ranga	5	PARA
Poecilia reticulata	6	POR

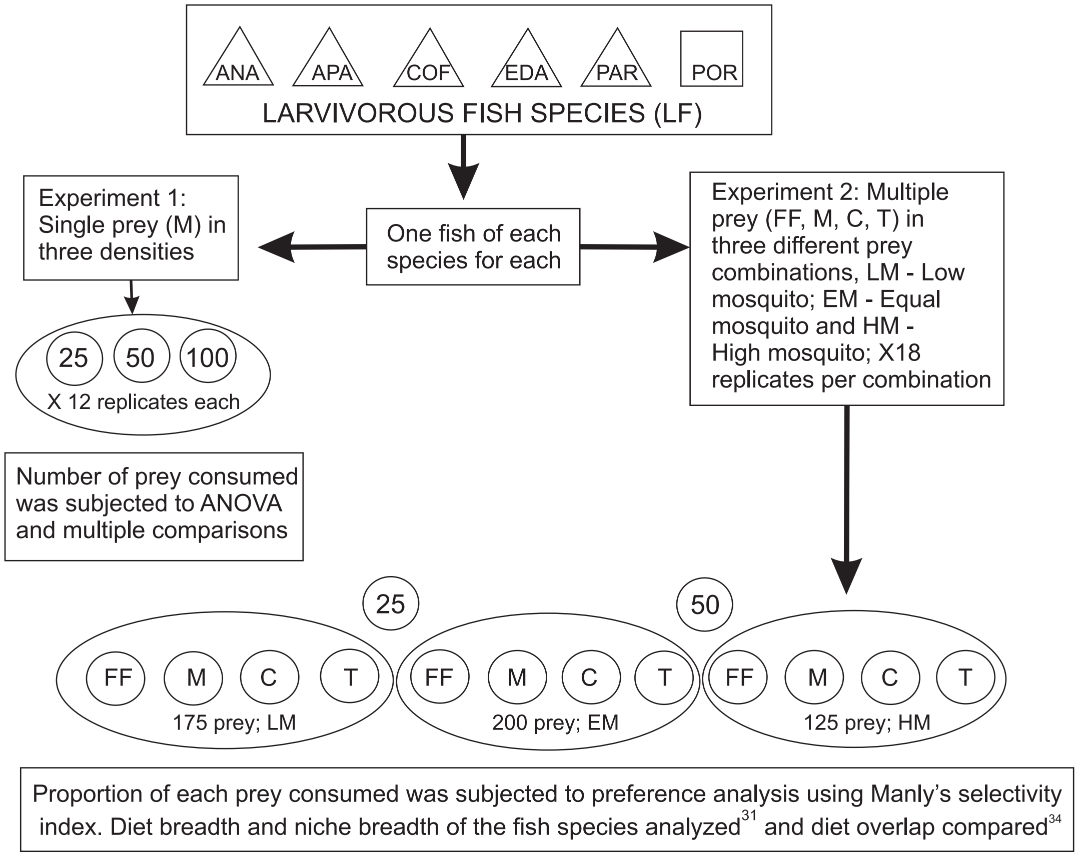

Fig. 1: Schematic representation of the experiments carried out with five indigenous (triangled) and one exotic (squared) larvivorous fish species (ANA –*Ambassis nama*; APA – *Aplocheilus panchax*; EDA – *Esomus danricus*; COF – *Colisa fasciatus*; PAR – *Parambassis ranga*; POR – *Poecilia reticulata*) and food items (FF – Fish food; M – Mosquito larvae; C – Chironomid larvae; T – Tubificid worms).

Table 2. The relative number of different food types consumed by the larvivorous fishes in a 2 h feeding bout

Fish species	Fish food	Mosquito larvae	*Cx. quinquefasciatus* larvae	Tubificid worms
Equal relative density of mosquito				
P. reticulata	3 ± 0.66	7.33 ± 0.89	9.25 ± 0.96	12.5 ± 0.95
A. nama	1.42 ± 0.26	13 ± 1.40	10.17 ± 0.46	10 ± 0.69
P. ranga	5.33 ± 0.63	8.08 ± 0.67	12.58 ± 1.06	14.67 ± 0.41
C. fasciatus	5.58 ± 0.76	20.92 ± 2.84	28.67 ± 2.45	18.17 ± 2.24
E. danricus	9.58 ± 0.92	8.33 ± 0.68	18.33 ± 2.71	17.25 ± 1.09
A. panchax	14.92 ± 1.87	10.75 ± 0.99	11.58 ± 0.63	13.08 ± 1.42
Low relative density of mosquito				
P. reticulata	1.75 ± 0.30	3 ± 0.25	11.33 ± 1.03	18.08 ± 0.88
A. nama	2.25 ± 0.60	9.75 ± 0.91	14.33 ± 0.92	14.33 ± 0.82
P. ranga	4.83 ± 0.58	5.42 ± 0.58	9.58 ± 1.10	11.75 ± 1.33
C. fasciatus	11.58 ± 0.97	7.58 ± 1.30	28 ± 3.98	16.33 ± 1.57
E. danricus	8.17 ± 1.34	3.33 ± 0.50	9.5 ± 0.94	17.17 ± 1.31
A. panchax	10.33 ± 0.62	13.50 ± 1.03	6.33 ± 0.38	19.50 ± 0.67
High relative density of mosquito				
P. reticulata	0.75 ± 0.28	8.08 ± 0.93	4.75 ± 0.70	4.58 ± 0.47
A. nama	1.5 ± 0.42	18.33 ± 1.84	10.92 ± 1.40	8.42 ± 1.28
P. ranga	2.17 ± 0.14	5.33 ± 0.40	8.92 ± 0.53	8.75 ± 0.25
C. fasciatus	2.08 ± 0.29	17.92 ± 2.31	20.67 ± 0.63	15.08 ± 1.59
E. danricus	8.08 ± 0.77	7.17 ± 0.76	8 ± 0.90	9.08 ± 0.71
A. panchax	2.92 ± 0.29	19.25 ± 0.71	5.17 ± 0.41	8.33 ± 0.68

Table 3. The value (mean ± SE) of selectivity exhibited by the larvivorous fishes, for the different food items, at equal, low and high relative number of mosquito prey, along with the results of *t*-statistics (for hypothesis tested, see text)

Prey ratio	Equal (50 M : 150)				Low (25 M : 150)				High (50 M : 75)			
Food item	FF	M	C	T	FF	M	C	T	FF	M	C	T
Expected selectivity value	0.25	0.25	0.25	0.25	0.29	0.14	0.29	0.29	0.2	0.4	0.2	0.2
P. reticulata												
t-value	**7.06**	0.93	1.52	***8.4***	**26**	1.56	0.77	***13***	**7.78**	**3.3**	***4.3***	***5.5***
LCL	0.05	0.18	0.23	0.35	0.03	0.14	0.26	0.45	0.01	0.23	0.26	0.27
UCL	0.14	0.28	0.28	0.42	0.07	0.19	0.19	0.52	0.09	0.37	0.37	0.37
Mean	**0.09**	**0.23**	**0.29**	**0.39**	**0.05**	**0.16**	**0.3**	**0.49**	**0.05**	**0.3**	**0.33**	**0.32**
SE	0.02	0.02	0.03	0.02	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.02
A. nama												
t-value	**31.5**	***3.7***	***3.9***	1.73	**17.2**	***8.4***	0.08	0.05	**9.51**	**2.73**	***6.5***	***2.5***
LCL	0.03	0.3	0.27	0.24	0.02	0.32	0.26	0.25	0.02	0.25	0.3	0.21
UCL	0.05	0.43	0.43	0.36	0.08	0.44	0.44	0.32	0.09	0.38	0.38	0.33
Mean	**0.04**	**0.37**	**0.3**	**0.3**	**0.05**	**0.38**	**0.28**	**0.29**	**0.05**	**0.32**	**0.36**	**0.27**
SE	0.01	0.03	0.01	0.03	0.01	0.03	0.01	0.02	0.02	0.03	0.02	0.03
P. ranga												
t-value	**8.44**	***2.8***	***2.5***	***7.8***	**9.85**	***4.8***	1.13	0.95	**5.87**	**33.4**	***11***	***14***
LCL	0.1	0.16	0.26	0.33	0.1	0.23	0.2	0.25	0.06	0.1	0.35	0.36
UCL	0.16	0.24	0.24	0.4	0.17	0.37	0.37	0.38	0.13	0.14	0.14	0.42
Mean	**0.13**	**0.2**	**0.31**	**0.36**	**0.13**	**0.3**	**0.26**	**0.31**	**0.1**	**0.12**	**0.39**	**0.39**
SE	0.01	0.02	0.02	0.01	0.02	0.03	0.03	0.03	0.02	0.01	0.02	0.01
C. fasciatus												
t-value	**18.2**	0.84	***4.3***	***0.2***	**6.47**	***2.1***	***2.4***	1.31	**28.9**	**8.46**	***19***	***3.9***
LCL	0.05	0.21	0.32	0.18	0.14	0.14	0.29	0.18	0.03	0.14	0.42	0.25
UCL	0.1	0.35	0.35	0.34	0.21	0.28	0.28	0.31	0.06	0.25	0.25	0.39
Mean	**0.08**	**0.28**	**0.39**	**0.26**	**0.17**	**0.21**	**0.37**	**0.25**	**0.04**	**0.19**	**0.44**	**0.32**
SE	0.01	0.03	0.03	0.04	0.02	0.03	0.04	0.03	0.01	0.02	0.01	0.03
E. danricus												
t-value	0.8	0.5	***3.9***	***5.7***	***3.7***	***26***	**12.4**	***6.5***	**3.73**	**19**	***2.6***	***4.8***
LCL	0.19	0.18	0.35	0.4	0.21	0.57	0.12	0.4	0.23	0.1	0.21	0.26
UCL	0.37	0.29	0.29	0.59	0.27	0.65	0.65	0.52	0.32	0.16	0.16	0.37
Mean	**0.28**	**0.24**	**0.48**	**0.49**	**0.24**	**0.61**	**0.15**	**0.46**	**0.28**	**0.13**	**0.28**	**0.32**
SE	0.04	0.02	0.06	0.04	0.01	0.02	0.01	0.03	0.02	0.01	0.03	0.02
A. panchax												
t-value	1.34	***2.2***	1.29	0.36	**17.1**	***15***	**24.5**	***2.1***	**8.44**	1.58	0.21	***6.2***
LCL	0.22	0.18	0.2	0.21	0.15	0.38	0.09	0.28	0.09	0.34	0.17	0.28
UCL	0.37	0.25	0.25	0.31	0.18	0.46	0.46	0.34	0.13	0.41	0.41	0.36
Mean	**0.3**	**0.21**	**0.23**	**0.26**	**0.16**	**0.42**	**0.1**	**0.31**	**0.11**	**0.37**	**0.2**	**0.32**
SE	0.03	0.02	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.02

FF–Fish food; M–Mosquito larvae; C–Chironomid larvae; T– Tubificid worms; UCL–Upper confidence limit; LCL–Lower confidence limit; SE–Standard error; *t*-values with bold are significant for lower than expected proportion and bold italics for higher than expected proportion; rest insignificant at $p < 0.05$; $t_{tab} = 1.796$.

the different combinations of food provided (Table 4) which may possibly an indication of species-specific adjustment of feeding habit depending on the relative proportion of controphic species present in the environment. It was observed that preference for mosquito larvae was influenced by the alternative food types and remained unaltered in most of the replicates for most of the fishes. The overall niche overlap based on the food type consumed at equal density was 0.933, calculated with 1000 simulations on the original data, with the help of EcoSim[24] (Table 5). This indicates that the larvivorous fishes consumed the food types in a similar manner and the difference, if any, was due to the preference pattern for a particular prey type.

Table 4. Values (mean ± SE) of the diet breadth and niche breadth of the indigenous larvivorous fishes and *P. reticulata*

Fish species	Niche breadth	Diet breadth
Equal relative density of mosquito		
P. reticulata	0.80 ± 0.03	0.74 ± 0.04
A. nama	0.75 ± 0.01	0.67 ± 0.01
P. ranga	0.84 ± 0.02	0.80 ± 0.03
C. fasciatus	0.76 ± 0.04	0.68 ± 0.05
E. danricus	0.82 ± 0.02	0.75 ± 0.02
A. panchax	0.91 ± 0.03	0.88 ± 0.04
Low relative density of mosquito		
P. reticulata	0.69 ± 0.02	0.64 ± 0.02
A. nama	0.75 ± 0.03	0.7 ± 0.03
P. ranga	0.83 ± 0.02	0.80 ± 0.03
C. fasciatus	0.81 ± 0.02	0.77 ± 1.02
E . danricus	0.78 ± 0.02	0.75 ± 0.03
A. panchax	0.78 ± 0.02	0.73 ± 0.02
High relative density of mosquito		
P. reticulata	0.79 ± 0.03	0.74 ± 0.03
A. nama	0.80 ± 0.02	0.75 ± 0.03
P. ranga	0.71 ± 0.02	0.63 ± 0.02
C. fasciatus	0.71 ± 0.02	0.64 ± 0.02
E. danricus	0.81 ± 0.03	0.77 ± 0.04
A. panchax	0.86 ± 0.29	0.82 ± 0.02

DISCUSSION

From the results it is evident that native larvivorous fishes *A. nama*, *A. panchax*, *C. fasciatus*, *E. danricus* and *P. ranga*, can consume considerable numbers of IV instar larvae of *Cx. quinquefasciatus*. Contrast to these fish species, *P. reticulata* consumed low numbers of mosquito larvae (Fig. 2). The number of mosquito prey consumed was dependent on number available and varied among fish species. The interaction between fish species and mosquito density indicates species-specific differences in predatory behaviour. This can be attributed to the variations in size and morphological features of the fishes.

In the presence of multiple alternative food items, *P. reticulata* exhibited significantly lower preference for mosquito larvae, while *A. panchax* showed higher than expected preference for mosquito larvae, similar to earlier studies[17, 18, 25]. For other fish species, the preference was poor for mosquito larvae. However, the difference in selectivity pattern can partly be attributed to size of the fishes that varied greatly among the fish species. The mouth gape of the larvivorous fishes differed among the species possibly contributed to the difference in the prey seizing pattern for mosquito and chironomid larvae in contrast to fish food and the tubificid worms. The mosquito and chironomid larvae were captured individually in contrast to the tubificid worms, which were caught in groups by the fishes, irrespective of sizes and species. The significantly lower selectivity value for mosquito larvae at higher proportions can also be due to crowding effect, since mosquito larvae remained in aggregate on the water surface. Except for *A. panchax* and *P. reticulata*, all the fish species oriented at the middle column (~ 6–8 cm above the bottom) of the aquaria. This spatial orientation of the prey and predators did not change even when the mosquito larvae were present at higher proportions. Earlier studies using *A. panchax* as predator demonstrated that the habitat conditions along with alternative prey influence mosquito prey consumption[17].

The present study, however, shows that selectivity of *Cx. quinquefasciatus* by this fish species may be affected by variations in relative density in comparison to alternative prey. The exotic fish *P. reticulata* failed to exhibit significant preference for mosquito larvae even at higher

Table 5. The relative consumption rate of different food type (A) and the similarity coefficient matrix of resource utilization (B) by different indigenous and exotic larvivorous fish species based on niche overlap model. The overall similarity value is 0.933, greater than the simulated (1000 repeats) value 0.857 significantly at $p < 0.001$ level

Food type/Fish species	Fish species					
	P. reticulata	*A. nama*	*P. ranga*	*C. fasciatus*	*E. danricus*	*A. panchax*
(A)						
Fish food	3	1.4	5.3	5.58	9.58	14.92
Mosquito larvae	7.3	13	8.08	20.92	8.33	10.75
Chironomid larvae	9.3	10.2	12.58	28.67	18.3	11.58
Tubificid worms	12.5	10	14.67	18.17	17.25	13.08
(B)						
P. reticulata		0.943	0.994	0.943	0.966	0.89
A. nama			0.926	0.967	0.881	0.834
P. ranga				0.949	0.988	0.919
C. fasciatus					0.94	0.86
E. danricus						0.938

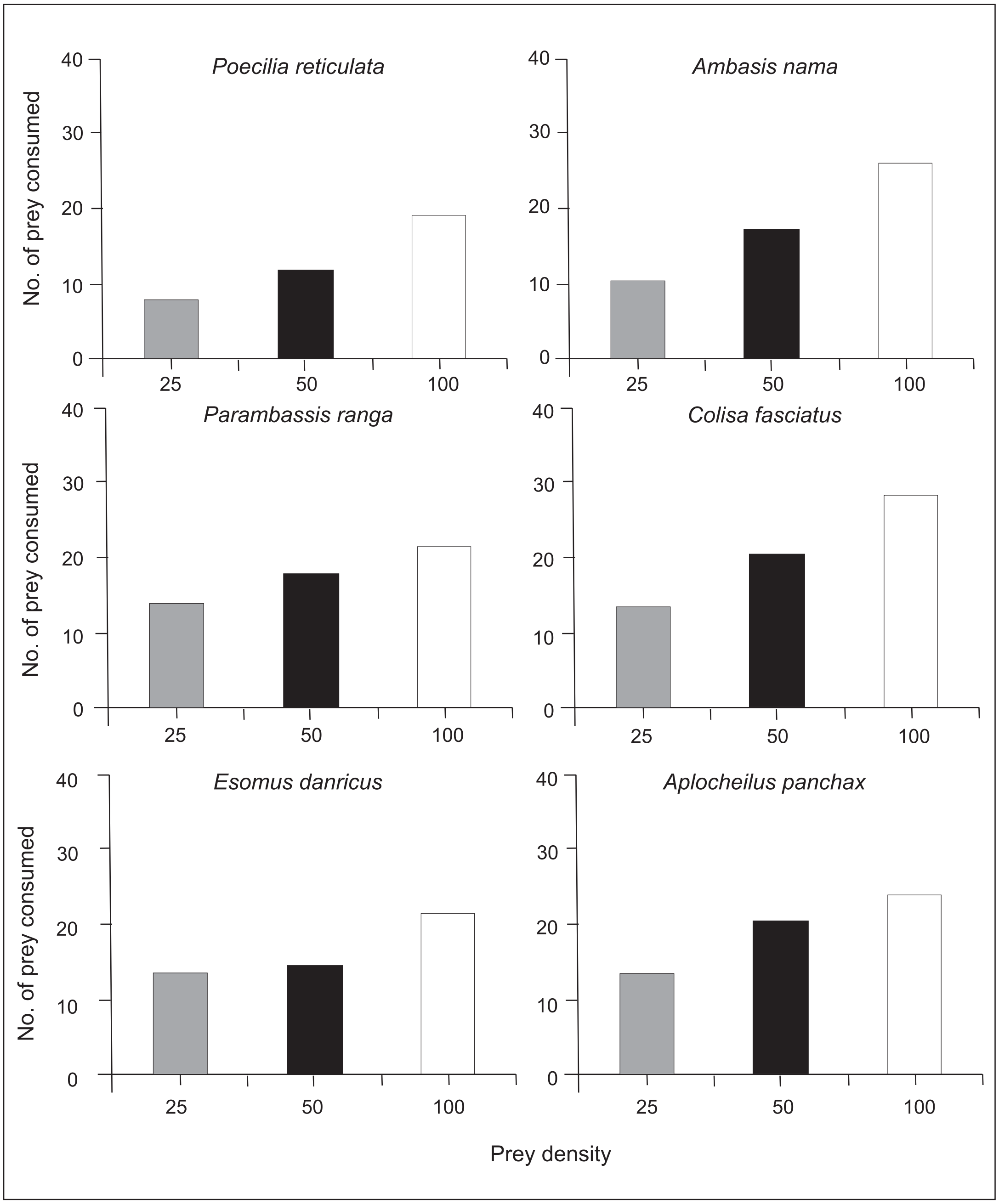

Fig. 2: The number (Mean ± SE) of IV instar *Cx. quinquefasciatus* larvae consumed by the larvivorous fishes at three different prey densities (n=12 replicates per fish species per prey density).

relative density (Table 3), similar to the earlier findings[18]. Lack of selectivity for *Cx. quinquefasciatus* larvae in the presence of alternative prey suggests that *P. reticulata* may be less effective in regulating mosquitoes in wetlands where multiple prey types are available. Although the numbers of mosquito larvae consumed in the presence of alternative food items remained within expected limits on few instances, the efficacy of the indigenous larvivorous fishes *A. nama*, *P. ranga, C. fasciatus*, *E. danricus* and *A. panchax*, were influenced by the alternative food types, particularly the live prey. Pertinent observations on consumption of non-target insects by mosquito fish *G. affinis*[5, 6] support this prediction. However, *P. reticulata* and other indigenous larvivorous fish may be comparatively more effective in regulating mosquitoes in habitats with insufficient alternative prey population. The successful regulation[2, 3] of mosquito by *P. reticulata* and *G. affinis* in village wells and similar mosquito larval habitats supports this proposition.

Alternative prey influences efficacy of biocontrol agents, evident from the studies on cyclopoid copepod[26], dytiscid beetles[27, 28], odonate larvae [29], larvae of the predatory mosquito *Toxorhynchites splendens*[30], and heteropteran bugs[31–33]. Although the mosquito prey consumption of the larvivorous fish remain higher than these insect predators, the impact of multiple alternative food types is obvious. Similar impact of alternative prey on mosquito prey selectivity is noted for *Cnesterodon decemmaculatus*, a native fish of South America. *Cnesterodon decemmaculatus* exhibits positive preference for cladocerans, copepods and chironomid larvae over mosquito larvae[34], indicating that the relative abundance of alternative prey influence selectivity and thus vulnerability of mosquito larvae. In case of the indigenous fishes, similar inference can be deduced, since selectivity of *Cx. quinquefasciatus* varied with the relative density of the alternative and target preys. Thus, while suggesting the use of these indigenous larvivorous fishes for biological control of mosquitoes, affinity for alternative prey may be a constraint to achieve satisfactory regulation, particularly for wetlands and allied habitats where multiple prey species are common. Further, wetland habitats are structurally complex owing to vegetations that impede prey capture by mosquito predators. Prey selection by indigenous larvivorous fish species may be complex under such conditions, as evident from the studies on *A. panchax* and indigenous fishes of Australia. Therefore, assessment of the efficacy of the indigenous larvivorous fish species under varying habitat conditions should be carried out prior to promoting their use in mosquito control programme.

The results of the present study indicate that the fish species *A. nama*, *A. panchax*, *C. fasciatus*, *E. danricus P. ranga* and *P. reticulata* failed to exhibit a definite and consistent selectivity pattern for mosquito larvae, irrespective of relative density, in the presence of alternative food items. It seems that under natural conditions, these fish species may consume mosquitoes to a lesser extent than expected since dietary requirements may be fulfilled by the alternative prey. Such dietary choice may facilitate survival and perpetuation of these fish species under situations where the target mosquito larvae will be low or extinct. The ecological basis of biological control demands this criterion of a biological resource for regulating target organisms[35]. Further, employing indigenous larvivorous fish species in mosquito regulation can support conservation of natural biota. Therefore, use of these and other indigenous larvivorous fishes considered in the present study may be a suitable option in regulating mosquitoes in diverse larval habitats. However, under field conditions, the multiple prey and predators[36] and habitat heterogeneity may impact the interactions between mosquito prey and fish predator. Possible consequences of such impact should be evaluated to further strengthen the use of these fishes alone or as a part of integrated vector mosquito control.

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Correspondence to: Dr Gautam Aditya, Ecology Laboratory, Department of Zoology, The University of Burdwan, Golapbag, Burdwan–713 104, India.
E-mail: gautamaditya2001@gmail.com; s.pal.bu@gmail.com; nabaneetasaha@gmail.com; gkszoo@rediffmail.com

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