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Functional responses of *Laccotrephes griseus* (Hemiptera: Nepidae) against *Culex quinquefasciatus* (Diptera: Culicidae) in laboratory bioassay

Anupam Ghosh[1] & Goutam Chandra[2]

[1]*Department of Zoology, Bankura Christian College, Bankura;* [2]*Mosquito and Microbiology Research Unit, Department of Zoology, Burdwan University, Burdwan, India*

ABSTRACT

Background and objectives: In integrated vector management programmes, major emphasis is given on the application of biocontrol agents. In the present study, the hemipteran water bug, *Laccotrephes griseus* (Hemiptera: Nepidae*)* was evaluated to establish its functional response against the immature *Culex quinquefasciatus* Say in the laboratory.

Methods: Adult water bugs (*L. griseus*) were collected from different ponds in Bankura, West Bengal, India. Predation rate was determined in semi-field conditions. Functional response, regression equation analysis and rate of predation were also determined in the presence of an alternative prey.

Results: The long-term predation experiment (up to 30 days) revealed that *L. griseus* is a very good predator of mosquito larvae. The functional curve analysis of *L. griseus* exhibited a Type II functional response. Their handling times and coefficient of attack rates were 29.37 min and 2.17 respectively. Regression equations showed that consumption rate was directly proportional to prey and predator densities and inversely proportional to the search area. The selectivity index, niche width and food breadth were also determined.

Interpretation and conclusion: The present study revealed that *L. griseus* can consume a good number of mosquito larvae in the laboratory conditions. The long-term bioassay also indicates their predatory response against mosquito larvae in the semi-field conditions. The result of selectivity index, niche width and food breadth were also highest for *Cx. quinquefasciatus.* In these respects, the water bugs *L. griseus* are indicative of their release in the aquatic habitat where *Cx. quinquefasciatus* is the most dominant mosquito species.

Key words Bioassay; coefficient of attack rates; food breadth; handling time; niche width; selectivity index

INTRODUCTION

The biting nuisance and disease-vector significance of different species of adult mosquitoes have drawn considerable attention to the means to control them. The reduction of immature or larval mosquitoes in aquatic habitat requires the use of bio-control agents rather than chemical insecticides to avoid ill effects on the aquatic community, to overcome the problems associated with the development of insecticide resistance & biological magnification and to preserve the natural faunal biodiversity of the aquatic ecosystem. The distinct advantages of bio-control agents are their ability to kill target species at relatively low doses, safe for non-target organisms including man, easy application in the field, inexpensive production, conservation of ecological diversity and little evidence of resistance development on target mosquitoes. Several aquatic insect predators, such as crustaceans[1], dytiscid beetles[2], notonectid bugs[3], odonate naiads[4] and the hemipteran bugs[5] were previously documented for their efficacy as mosquito control agents in laboratory as well as in the field conditions.

The description of a predator’s instantaneous, feeding rate or predatory impact, as a function of prey density, is its ‘functional response’. The functional response of a predator is a key factor in regulating the population dynamics of predator-prey systems in any ecosystem. It describes the rate at which a predator kills its prey at different prey densities and can thus determine the efficiency of a predator in controlling prey populations in biological control programmes in laboratory condition or in field survey[6]. The functional response of a natural enemy offers a good conceptual framework for understanding the action of agents in inundative releases in field also[7]. The functional response curves can be differentiated by evaluating the parameters, such as attack rate and handling time (time spent by predator in attacking, killing, subduing, and digesting the prey). The attack rate estimates the steepness of the increase in the rate of predation with increasing prey density, and handling time is very useful to estimate the satiation threshold.

Arthropod predators generally display one of the three types of functional responses. The functional response curves may represent an increasing linear relationship (Type I), a decelerating curve (Type II), or a sigmoidal relationship (Type III)[8].

In a Type-I response, the number of prey killed increases linearly at a constant rate up to a maximum and then remains constant with increasing prey density and can be estimated by a linear equation as: $N_e = \alpha+\beta n$, where N_e = number of prey eaten, N = Prey density, α and β are intercepts and slope of the equation respectively[9].

In a Type-II functional response, the number of prey eaten increases with prey density but after reaching a point (asymptote) hyperbolically it becomes inversely density dependent and can be estimated by Holling Disc Equation[10] as, $Ha = aHT/(1 + aHT_h)$, where 'a' is the attacking rate, 'T_h' the handling time, 'T' the total time of predation, 'Ha' total prey eaten, and 'H' the initial prey density[9].

In a Type-III functional response, the number of prey killed approaches asymptote as a sigmoid function and then decreases with increase in prey density and can be estimated by the following formula:

$a = d+Hn/ 1+cN$, where, a = attack rate, H = handling time, N = Number of prey, 'c' and 'd' are constants[9].

Laccotrephes griseus (Hemiptera: Nepidae) is a predaceous aquatic bug commonly called nepa or water scorpion. It inhabits the littoral zones of ponds and temporary water bodies[11]. Larval and adult stages of *L. griseus* coexist with the mosquito larvae and hence the former has been used as a tool for biological control[12].

The present study was designed to evaluate the predatory efficiency and functional response of the *L. griseus* on immature stages of *Cx. quinquefasciatus* (Diptera: Culicidae) as prey in the context of their presence in the same guild in their natural habitat. The results of the present study are expected to highlight the variation in predatory potentiality in the presence of alternative prey items and variation of predation rate with change in the search area and predator density during long time predation experiments.

MATERIAL & METHODS

Collection of L. griseus and mosquito larvae

Adult water bugs (*L. griseus*) were collected from different ponds in Bankura, a district located in the state of West Bengal in India during the month of May 2008 with the help of an insect net having 200 μm mesh size. Adult morphs of the predators (irrespective of sex) were identified from the Zoological Survey of India (Voucher specimen No. 63/2008), segregated in the laboratory and maintained within glass aquaria (30×20×20 inches) containing pond water (10 litres). Few specimens of aquatic weeds and gravels were placed inside the aquarium to simulate natural conditions. The insects were collected 7 days before the commencement of the experiments and were maintained in the laboratory for acclimatization with *Cx. quinquefasciatus* larvae as food. The average body length of the *L. griseus* used in the experiments was 19.2 ± 2.1 mm.

Mosquito larvae were collected from cemented drains of the same area. The III instar larvae to be used in the experiments were separated and kept in enamel trays with an adequate amount of food (Tokyo, Japan).

Predation rate in semi field condition during long-term bioassay

For a long-term study on predation rate, for 30 consecutive days, a single/two *L. griseus* was/were kept in a plastic bucket filled with 20 litres of pond water and 100 III instar *Cx. quinquefasciatus* larvae/day were given as food and the consumption rate was recorded after 24 h. After counting the number of consumed larvae on each day the same number of larvae were put in the water to maintain the same prey density.

Functional response analysis

To each adult specimen of *L. griseus*, III instar larvae of *Cx. quinquefasciatus* were supplied at densities of 10, 20, 30, 40, 50, 60, 70, 80 and 90 per 250 ml of a glass beaker and allowed to predate for a period of 24 h. Nine replicates for each of the prey densities were carried out for the determination of the rate of predation and the functional response. To determine the type of functional response, a non-linear polynomial regression equation was established taking into consideration the proportion of prey eaten (Na/No) as a function of prey offered (No). The equation describes the relationship between Na / No and No:

$$\frac{Na}{No} = \frac{\exp(P0 + P1No + P2No^2 + P3No^3)}{1 + \exp(P0+ P1No + P2No^2 + P3No^3)}$$

Where, P0, P1, P2, and P3 are the intercept, linear, quadratic and cubic coefficients, respectively. If P1 >0 and P2 <0, the proportion of prey consumed is directly proportional to the prey density, thus, describing a Type III functional response. If P1 <0, the proportion of prey consumed declines monotonically with the initial number of prey introduced, thus, describing a Type II functional response[8,13].

After the determination of the shape of the curve (Type-II response), the handling times and attack coefficients of a Type II response were analyzed by curvilinear function as Holling Disc equation[10]. The attack rate and the handling time used to establish the equation of the functional response, were determined after linearization of the Holling Disc Equation[10], according to, $1/Ha = (1/a)/ (1/HT) + T_h/T$ equivalent to $y = \alpha + \beta x$[14]. Thus, the handling period of prey (Th) can be determined with simple linear equation by plotting the data of H/Ha versus H and multiplying the total exposition time (T) by the angular coefficient of this straight line (β). The attack rate (a) corresponds to the intercept of the straight line ($1/\alpha$)[15].

Regression equation analysis

In this experiment, 40 and 80 *Cx. quinquefasciatus* larvae per 250 and 500 ml respectively of water were provided to a single or two *L. griseus* for a period of 24 h for regression equation analysis. The relationship of feeding rate (Y) with changes in the predator numbers (X_1), water volume (X_2) and prey densities (X_3) was expressed by least square regression equation analysis using Microsoft Excel (MS Office 2000). This multiple regression of Y, X_1, X_2 and X_3 gives first order relationship with variables. Six replicates on separate dates were carried out to establish the regression equation.

Rate of predation in the presence of an alternative prey

A total of 40 preys (10 *Anopheles subpictus*; 10 *Cx. quinquefasciatus*; 10 *Armigeres subalbatus* and 10 *Stegomiya aegypti* larvae) were placed in a 250 ml glass beaker to an adult individual of *L. griseus* for a period of 24 h to know the prey preference of the predator species. The collected data were subjected to prey selectivity analysis following Krebs[16] and Rehage *et al*[17]. The formulae used in the study were: $w_i = c_i / a_i$,

Where, w_i = prey preference of the predator, c_i = proportion of the ith prey consumed by the predator, a_i = proportion of the ith prey available during experiment and $S_i = w_i / \sum w_i$, where Si = selectivity index. The niche breadth 'N' and the food breadth 'B' were determined as,

$$N = 1/ \sum (ci^2/ai) \text{ and } B = (N-a_{min})/ (1-a_{min})$$

Where, a_{min} is the lowest proportion of prey type available during the experiment[4]. The experiment was repeated six times to draw a definite conclusion about the above parameters. During each of the experiment the predators were fed to satiation and then starved for 24 h before their utilization in the experiments to equalize the hunger level. The pond water was collected from the same aquatic habitat to that of the predators. The water pH ranged from 7.8–8.3 and the water temperature from 29 to 36°C.

RESULTS

During the course of the experiments, observations were made on the predatory behavior of *L. griseus.* These insects chased the preys horizontally below the surface of water. After a successful encounter with prey, *L. griseus* grasped a larva with its pro and mesothoracic legs, subdued the struggling larva by puncturing its body with the help of the sharp rostrum, and then fed on its internal body fluid. Finally, the dead remains of the larva, including mainly the sclerotized head, were discarded and the predator hunted for its next prey. After the consumption of a single larva they usually move vertically through the water and after a few moments reach its surface in search of prey.

The results of the long-term bioassay experiment indicated that the rate of predation for a single individual exists between a lowest of 21 larvae/day (on the 5th day) and a highest of 51 larvae/day (on the 17th day). On the other hand, lowest predation (38 larvae/day) was noticed on the 18th day and the highest value of 74 larvae/day was noticed on 21st day in case of the predation experiment for two individuals (Fig. 1). Although the average rate of predation increased with predator density, the increase was not linear. During the experiment, when two individuals were put into the same experimental bucket, it was noticed that they marked the whole water surface into two distinct and almost equal halves as their own separate territories and when one individual entered the half of the other, they often clogged each other and fought to restore their respective territory. Their predatory efficacy, therefore, might have been reduced.

The functional curve analysis of *L. griseus* exhibited an initial sharp increase with increase in prey density, i.e. *L. griseus* responded more vigorously at lower prey densities and then there was a decline in consumption rate at higher densities (Fig. 2). Polynomial regression equation analysis estimated the P0 value of –2.536 (Standard error, 1.206), P1 of –0.006 (Standard error, 0.99), P2 value of –9.653 (Standard error, 0.002) and P3 value of 6.715 (Standard error, 0). The R^2 value is 0.896. This suggests that *L. griseus* exhibited a type II functional response as $P1<0$. The slope and intercept of the Holling Disc Equation were 0.02 and 0.46 respectively and their corresponding handling times and coefficients of attack rates were 29.37 min and 2.17 respectively.

Variation in the larval consumption rate with variation of prey densities and search area was presented in Table 1. From the established regression equation it was noticed that consumption rate was directly proportional to prey and predator densities and inversely proportional

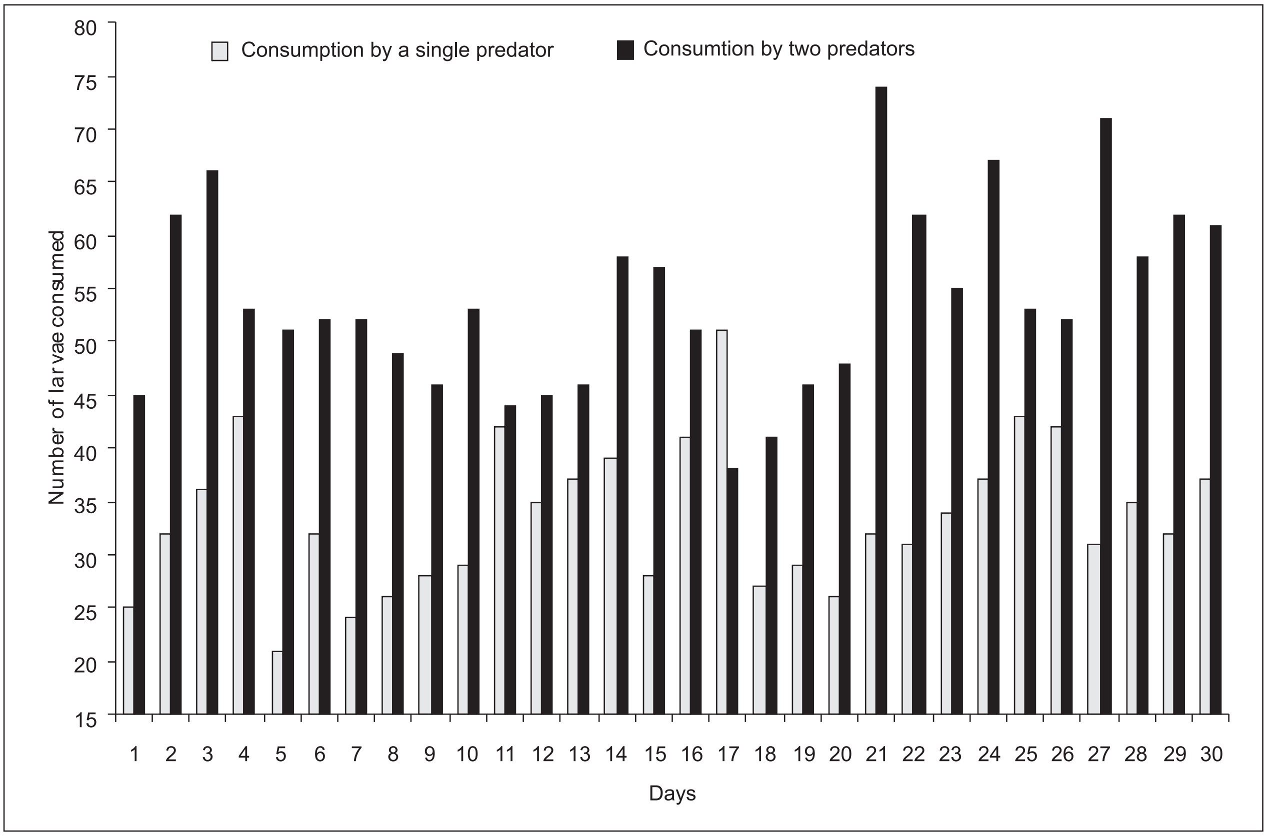

Fig. 1: The results of the long-term bioassay experiment with single/two predator(s) for 30 consecutive days.

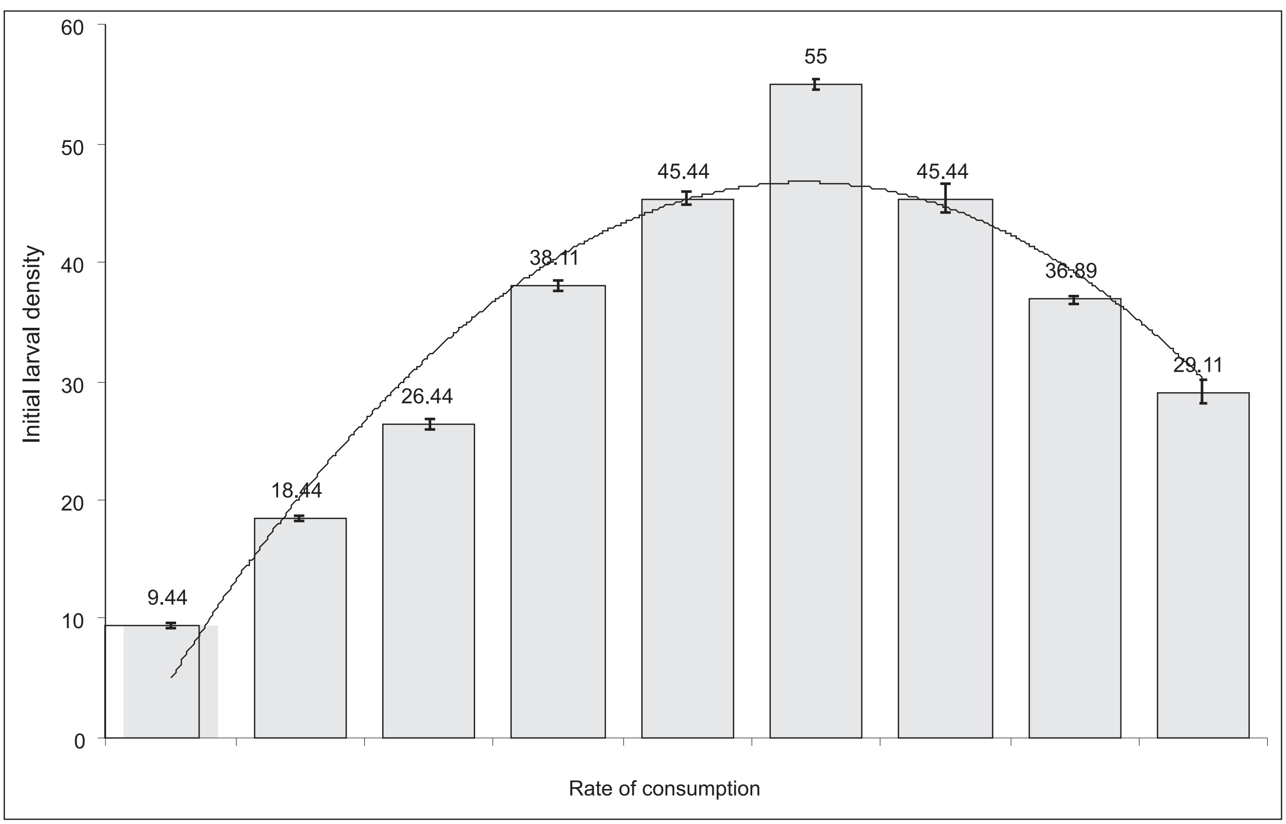

Fig. 2: The functional curve analysis of *L. griseus* against *Cx. quinquefasciatus* larvae.

Table 1. Regression equation analysis of *L. griseus* against *Cx. quinquefasciatus* in variable prey density and search area (n = 3) (observations per prey density and search area)

No. of predators (X_1)	Search area (X_2) (ml)	Initial larval density (X_3)	Rate of consumption (Y) (Mean ± SE)	Regression equation	R^2 value
1	250	40	28.66 ± 1.45	$Y=3.165+4.25X_1-0.177X_2+0.652X_3$	0.996
		80	55.66 ± 3.53		
	500	40	24 ± 2.52		
		80	51.33 ± 1.45		
2	250	40	33 ± 1.53		
		80	59.66 ± 1.20		
	500	40	30.33 ± 1.20		
		80	53.66 ± 1.45		

Table 2. Selectivity index, niche width and food breadth analysis of *L. griseus* in the presence of different mosquito species (n=3 observations)

Responses	Mosquito larvae selected			
	Stegomyia aegypti	*Anopheles subpictus*	*Culex quinquefasciatus*	*Armigeres subalbatus*
Predatory efficiency (No. of consumed larvae/day)	8±0.58	8.66±0.33	9.33±0.33	3.66±0.33
Selectivity index	0.2698	0.292	0.3147	0.1235
Niche width	0.9177			
Food breadth	0.89			

to the amount of water volume (search area) for an individual predator.

The results of selectivity indices for different prey types, niche width and food breadth are presented in Table 2. Result of paired *t*-test revealed that selectivity index was highest for *Cx. quinquefasciatus* and it is statistically significant (p <0.05) from *Ar. subalbatus* (t = 6.396) but statistically insignificant (p >0.05) from *An. subpictus* (t = 1.428) and *S. aegypti* (t = 1.21) against the table value of 2.776 with degrees of freedom 4.

DISCUSSION

Laccotrephes griseus was previously documented for its mosquitocidal activities. These insects feed by piercing the prey with the rostrum, injecting digestive juices, and sucking the liquid contents from the prey. These aquatic macro-invertebrate predators are reported to coexist abundantly with other organisms; including the aquatic stages of several species of mosquitoes[12]. Earlier workers have investigated the biological control potential of the *L. griseus* at different prey densities and in different sexes[18-20]. But a detailed account on their numerical responses against the variable density of prey is needed before recommending their augmentative release in the field conditions.

Understanding the prey-predator relationship is an important aspect in community ecology, and one principal component of this relationship is the predator's rate of feeding upon the prey. The feeding rate or predatory impact describes the transfer of biomass between trophic levels and depends upon the habitat complexity and prey/predator densities. Functional response of a predator against prey is the mathematical form of the feeding rate that can influence the spatio-temporal variation of predators, correlations between nutrient enrichment and the biomass of higher trophic levels[21], and the length of food chains[22]. During the present study, the prey-predator relationship exhibited a type II response that describes the average feeding rate of a predator when it spends some time searching for the prey and some time, exclusive of searching, processing each captured prey item (i.e. handling time) and also some time engaging in encounters with other predators.

The present study revealed that *L. griseus* can consume a good number of larvae in the laboratory condition when the larval density ranges below 280 larvae/l (70 larvae/250 ml) of water, as increase in the larval density over this value causes a cessation in the feeding rate (Type II response). The study also provides a preliminary idea about the change in the predation efficiency with change in the larval density and search area from established regression equation. The regression equation analysis is a very effective parameter in the determination of actual feeding rate in the field condition as from the established regression equation it is possible to determine the number of predators that should be introduced in response to a particular per dip prey density and available volume of search area in the field. Predator potentiality of coupled predators was considerably less than twice as much as that of an individual predator, which may be attributed to intraspecific interferences such as mounting of one individual over another during the course of the experiment. Habitat specificity and their prey preferences are also determined – they act as important criteria for selection of and qualification as a potential biological control agent. The long-term bioassay also indicates their predatory response against mosquito larvae in the semi-field conditions. Habitat specificity, functional response, prey preference and abundance in nature are important criteria for selection and qualification as a potential biological control agent. In this respect the water bugs *L. griseus* are indicative of their release in the aquatic habitat where *Cx. quinquefasciatus* is the most dominant mosquito species.

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Correspondence to: Dr Goutam Chandra, Professor, Department of Zoology, Mosquito and Microbiology Research Unit, Parasitology Laboratory, The University of Burdwan, Burdwan-713 104, India.
E-mail: goutamchandra63@yahoo.co.in

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