Functional response analysis of *Anisops sardea* (Hemiptera: Notonectidae) against *Culex quinquefasciatus* in laboratory condition

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Background & objectives: Culex quinquefasciatus is the principal vector of lymphatic filariasis (LF). Application of alternative vector control methodologies are aimed at reduction of mosquito breeding sites and biting activity through the use of biological control methods. In the present study, functional response of aquatic Hemipteran backswimmer, *Anisops sardea* was assessed against *Cx. quinquefasciatus* larvae in laboratory bioassay.

Methods: The functional respons of *A. sardea* was assessed against IIIrd instar larvae of *Cx. quinquefasciatus*. Respective handling times and coefficient of attack rates were determined by a non linear polynomeal regression equation.

Results: The results of rates of predation in variable prey densities exhibited a 'linear rise to plateau curve', associated with 'Type -II' functional response. The logistic regression estimated a significant negative linear parameter (P_1 <0) which also supported the same observation. Associated 'attack rates' and 'handling times' were also calculated using the Holling Disc Equation.

Interpretation & conclusions: The results of present experiments indicate that *A. sardea* can be used as a biocontrol agent against the larval forms of *Cx. quinquefasciatus* in temporarily available breeding places of mosquito with relatively clear water. However, a detailed field study has to be done to confirm these findings.

Key words Anisops sardea - consumption rate - functional response - habitat modification - mosquito larva

Mosquitoes are vectors of many protozoan, viral, and nematode-associated diseases that affect public health. *Culex quinquefasciatus* is the principal vector of lymphatic filariasis (LF). A current estimate reveals that about 120 million people in 83 countries are infected with LF parasites and more than 20 per cent of the world population are at risk of acquiring filarial infection. In India it is estimated that about 554.2 million people are at risk of LF infection in 243 districts¹.

Vector control strategies (either as adulticides or larvicides) include application of chemical, environmental, genetical, biological or integrated control avenues. Considering the better health of ecosystems, biodegradable nature, negligible evidences of resistance development in target species, minimum adverse effect on non-target organisms including human beings and to preserve the faunal biodiversity, the biological control methodology is now widely accepted and practiced throughout the world. There are various options available for biocontrol of immature mosquitoes in temporary and permanent water bodies, such as use of amphibian tadpoles, fishes, predatory insects, cyclopoid copepods, helminthes, *etc.* Among the predatory insects, the nymphal forms of dragonfly (Odonata: Zygoptera) and damselfly (Odonata: Anisoptera) are widely used in mosquito control studies due to their wide distribution, species diversity and trophic interactions²⁻⁴.

sardea Herrich-Schaeffer (Hemiptera: Anisops Notonectidae) is a small-bodied aquatic backswimmer found in high densities in many temporary pools and permanent water bodies throughout India. Laboratory based predatory experiments have revealed that this species have a high predation rate against larval Culex mosquitoes^{5,6}. The size selective prey preference of A. sardea against Daphnia was reported by Lindholm and Hessen⁷. Its effects on oviposition habitat selection of mosquitoes and other dipterans and on community structure have been studied⁸. Before recommendation of any species in field, a detailed numerical analysis of the predator species against variable prey densities and suitable habitat condition is necessary. Therefore, in the present study an attempt was made to analyze the functional responses of this species against Cx. quinquefasciatus, the most common filarial vector in South East Asia.

Material & Methods

This study was conducted in the department of Zoology, Bankura Sammilani College, Bankura, West Bengal, India.

Collection of prey and predator species: Adult predator species, viz. A. sardea, were collected from the different water bodies of Bankura, West Bengal, India, during May-June 2008, with an insect net having 200-µm mesh size. From the collected predator populations, 50 organisms of almost similar size and morphology were separated and kept in five glass beakers (1 1 capacity) containing pond water in the laboratory of Department of Zoology, Bankura Sammilani College. From each of these beakers one organism was collected randomly and altogether five adult organisms were identified from the Zoological Survey of India (ZSI, Voucher specimen No. 62/2008). Each of the specimens was identified as *A. sardea* by ZSI based on available scientific literatures. However, male/female identifications were not carried out. Specimens of aquatic weeds and gravels were placed inside the aquarium to produce natural conditions. The average body length of *A. sardea* used in the experiments was 0.6-0.7 mm.

Mosquito larvae were collected from cemented drains of the same area at regular intervals during the experiments as required. After each collection, the third instars larvae of *C. quinquefasciatus* were separated from the other larval instars based on length and maturity and kept within enamel trays in the laboratory with an adequate amount of food. Third instars larvae were used during the experiments as they are easily visible that make count easy and there is no chance to metamorphose into a pupa unlike fourth instars. The specific instars were identified following the key of Chandra (2000)⁹ which was prepared based on Christophers (1933)¹⁰ and Barraud (1934)¹¹.

The predatory insects were collected 10 days before the commencement of the experiments and were maintained in the laboratory for acclimatization with *Cx. quinquefasciatus* larvae as food.

Functional response analysis: To each adult specimen of *A. sardea*, IIIrd instar larvae of *Cx. quinquefasciatus* were supplied at various prey densities (10, 20, 30, 40, 50, 60, 70, 80 and 90 larvae per 500 ml of pond water) in glass beakers (11) and were allowed to predate for a period of 24 h within a BOD incubator (Eastern Instrument, India 180cft volume) at a temperature of 34° C (temperature found in the laboratory during the study period), humidity of 80-86%, and photoperiod of 14h L: 10h D.

The analysis of the functional responses was carried out in two steps according to Juliano¹²: *(i)* determination of the type of functional response by nonlinear polynomial logistic regression equation between proportions of prey consumed and given initial prey density; and *(ii)* estimation of the functional response parameters. The nonlinear polynomial regression equation¹³ used was as follows:

$$\frac{N_a}{N_0} = \frac{\exp\left(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3\right)}{1 + \exp\left(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3\right)}$$

Where N_a is the number of prey eaten, N_0 is the initial number of prey given, P_0 , P_1 , P_2 , and P_3 being the intercept, linear, quadratic and cubic coefficients,

respectively. If the linear coefficient is significant and negative *i.e.*, $P_1 < 0$, then the data describe a type II functional response curve¹³.

In the second step, the functional response parameters were estimated through a nonlinear regression procedure (NLR; SPSS 2006; IBM). The Holling's Disc equation¹⁴ was used to estimate the parameters. The associated equation was

$$N_a = \frac{aN_0T}{1 + aN_0T_k}$$

where N_a is the number of prey eaten, N_0 is the initial number of prey, *a* is the attack constant, *T* is the total time available (24 h) and T_h is the handling time per prey¹³.

Nine replicates for each of the prey densities were carried out with nine different adult morphs with similar size for determination of the rate of predation and the type of functional response. During each of the experiments 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 aquatic habitats common to predators.

Results & Discussion

The results of rates of predation in variable prey densities are presented in the Figure. Associated regression equation and 'R' value are also presented in the same figure. The results exhibited a 'linear rise to plateau curve' as the estimated rate of predation increased with initial prey density until it reached the upper asymptote and thereafter remained nearly unchanged or showed inverse density-dependence. Associated intercept, linear, quadratic and cubic coefficient values were given in Table I. The predator species exhibited a 'Type-II' functional curve as the logistic regression estimated a significant negative linear parameter (P1 < 0). Type-II nature of the functional curve was further confirmed from the Figure, where proportion of prey consumed was plotted against given initial prey density. So, the Holling Disc Equation can be used for the estimation of instantaneous attack rate (a) and handling time (T_h) . The associated attack rates and handling times are presented in Table II.

The functional response of predators against prey describes how the consumption rate of individual consumers changes with respect to resource density over a given time interval^{15,16}. The functional responses showed by predators against variable density of prey are of three basic types. It may of Type I, which represents an increasing linear relationship, Type II, that exhibits a decelerating curve, or Type III, that shows a sigmoidal relationship. In most of the single predator-single prey experiments functional response curves associated with insect predators' exhibits a Type II response^{6,15}.



Fig. Predation rate (mean \pm standard error) of *Anisops sardea* against *Culex quinquefasciatus* (n=9 experiments) with logarithmic trend line.

Parameters Estimates	Asymptotic Std. Error	Asymptotic 95% confidence interval	
		Lower	Upper
0.4955	0.2834	-0.2329	1.2239
-0.0539	0.0232	-0.1137	0.0058
0.0009	0.0005	-0.0004	0.0023
-6.6574E-06	3.4712E-06	-0.00001	2.2656E-06
	0.4955 -0.0539 0.0009 -6.6574E-06	0.4955 0.2834 -0.0539 0.0232 0.0009 0.0005 -6.6574E-06 3.4712E-06	Lower 0.4955 0.2834 -0.2329 -0.0539 0.0232 -0.1137 0.0009 0.0005 -0.0004 -6.6574E-06 3.4712E-06 -0.00001

Table II. Estimates (± SE) instantaneous attack rate and handling time of A. sardea against Cx. quinquefasciatus at variable prey densities Asymptotic Parameter Estimate Asymptotic 95% confidence interval Std. Error Lower Upper а 0.0357 0.0118 0.0077 0.0637 Th 1.0749 0.1793 0.6509 1.4989 R squared = 1 - Residual SS/Corrected Sum of Square = 0.78505

a, attach rate; T_h, handling time

Functional response has multi dimensional significance from the biological point of view. It works as a principal instrument for understanding the ecology between different kinds of predators and their prey items and depends upon the habitat complexity of an ecosystem. The present study revealed that *A. sardea* predated a good number of larvae in laboratory condition and exhibited a Type-II response like other insects.

The frequencies of mosquito borne diseases are ever increasing in the tropical countries associated with high mortality and morbidity. The highest incidence of these diseases mainly occurs during the rainy season because of creation of temporary water logging aquatic habitats in nature and household areas that act as a breeding ground of many mosquito species. In the clear aquatic habitats mainly, Anopheles mosquitoes breed, whereas polluted water logged areas act as breeding ground of Culex mosquitoes. Anisops species co-exist with Anopheles mosquitoes in clear aquatic habitats as well as field survey reported its existence with Culex mosquitoes¹⁶. Therefore, the augmentative release of the predator species in temporary habitats with polluted water where Culex mosquitoes breed is beneficial as evident from the result of the present study. It is also reported that Anisops releases a Kairomone that prevents oviposition of other mosquitoes¹⁷.

In conclusion, the findings of our study show that the application of *A. sardea* can be effectively used in temporary or permanent water logged habitat of different species of mosquitoes. However, a detailed field study is necessary before its wide application in mosquito control programme.

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