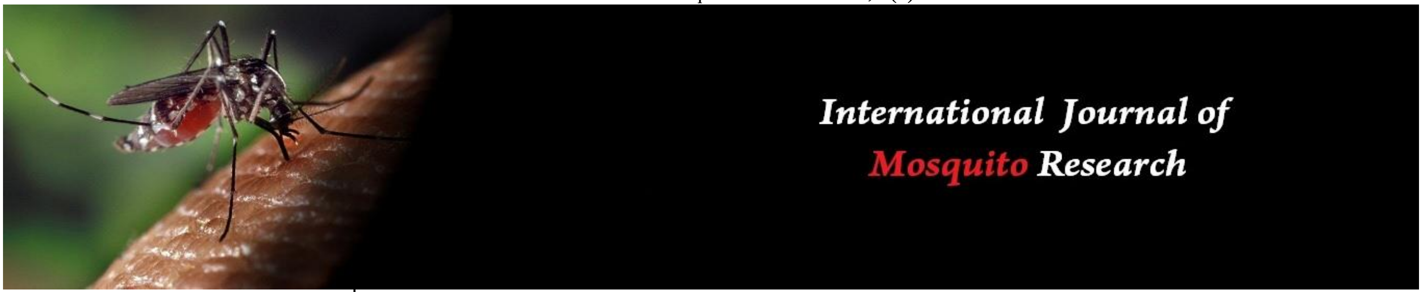

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Predation potentiality of *Notopterus notopterus* on JE vector, *Culex vishnui*

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Abstract
The present laboratory bioassay was designed to determine the predatory potentiality of the bronze featherback, *Notopterus notopterus* (Actinopterygii: Osteoglossiformes) against the larvae of *Culex vishnui*, vector of the zoonotic disease Japanese Encephalitis. The objectives of the present study includes: analysis of the functional response of the fish species against mosquito immature, determination of functional response parameters and the effect of habitat complexity on consumption efficacy of the predatory fish. The results of the investigation revealed that consumption rate of predator increased with time from the lowest number of 650 larvae on the 1^{st} day to 1039 larvae on 7^{th} day. The functional response was Type II having handling time of 0.15 min and attack rate of 2.95. Habitat heterogeneity also showed a significant effect on the consumption rate of the fish. The result of the study suggests that *N. notopterus* can be effectively used in fields to reduce the population of *Cx. vishnui*.

Keywords: attack rate, functional response, handling time, habitat heterogeneity, predation potential

Introduction

Mosquitoes are the vectors of many public health hazards like malaria, filariasis, dengue, encephalitis etc. Increasing occurrence of these ailments over the last few decades has necessitated application of efficient vector control strategies. The control of larval mosquitoes in aquatic habitats through the application of bio control agents is preferred in present day to avoid the development of insecticide resistance, biological magnification of toxic chemicals through food chain, toxic effect on non target organisms and environment and to preserve the natural biodiversity globally.

Among the various weapons of biological control of mosquitoes, such as application of aquatic insects [1-3], plant secondary metabolites [4], microbial biocides [5] etc; larvivorous fishes are the most common and affordable one. Different fish species such as *Aphanis dispar*, *Aplocheilus blocki*, *Colisa fasciatus*, *Chanda nama*, *Oryzias melastigma*, *Danio rerio*, *Macropodus cupanus* are important indigenous mosquito predators [6-7]. Whereas, among exotic species, *Clarias gariepinus* [8], *Gambusia affinis* and *Lebistes reticulta* [9], *Oreochromis niloticus* [10], *Xenentodon cancilla* [11], *Carrasius auratus* [12] etc. are important larvivorous fishes reported.

Functional response is an important factor in prey-predator interaction and it can evaluate the influence of a predator in changing the population dynamics of the prey. Functional response may be of different types like Type I, Type II, Type III, Type IV and Type V. Analyses of response parameters viz. predator's attack rate (a) and handling time (Th) against prey items are important to assess the type of response. The predator-prey interaction is influenced by prevailing habitat condition. Different researches have reported the changes in the predatory potentiality in presence of habitat heterogeneity, such as presence of aquatic vegetations [13-15] and altered photoperiod [16-17] etc. In view of the above, the present study was designed to evaluate the predatory efficiency of a fish species *Notopterus notopterus* Pallas, 1769 on immature stages of JE vector, *Culex vishnui* as prey in laboratory based bioassay. *N. notopterus* is a carni-omnivorous, euryphagic and bottom feeder fish [18]. Present study includes the functional response analysis and determination of handling time and attack rate of *N. notopterus* against *Cx. vishnui* larval mosquitoes. The objective of the present study also includes the influence of habitat heterogeneity viz. effect of vegetation, type of experimental medium and light on predatory potentiality of the predator fish.

2. Materials and Methods
2.1. Collection of fishes and mosquito larvae
A living freshwater fish, with a length of 14.4 cm and weight of 19.1 gm was collected from a fisherman of Burdwan, West Bengal, India, in the month of July 2016. This fish was identified as *Notopterus notopterus* through literature survey. It was maintained within glass aquarium (30×20×20 cm) containing pond water in the laboratory of Mosquito Research Unit, Department of Zoology, The University of Burdwan. Few specimens of aquatic plants such as *Chara* and *Vallisneria* were introduced into the aquarium to simulate natural conditions. The fish was acclimatized in the laboratory for 15 days before the commencement of the experiments with artificial food.
Mosquito larvae were collected from rice fields of the same area. The 3rd instar larvae were separated from the heterogeneous mixture of different instars of larvae by proper sieving and visual identification and kept in enamel trays with adequate water and food (mixture of dog biscuits and algae in 1:1 ratio) for experimentation.

2.2. Laboratory bioassay
For a short term study on predation rate, a single *N. notopterus* was kept for 7 consecutive days in a glass aquaria filled with 15 L of tap water (pH, 6.5) and 2000 third instar *Cx. vishnui* larvae/ day were given as food and the consumption rate were noted after every 24 hour. After counting the number of consumed larvae on each day, the same number of larvae was replenished in the aquarium to maintain the same prey density with sufficient amount of larval food.

2.3. Influence of Habitat Heterogeneity
To observe the changes in predatory potentiality and to include the habitat heterogeneity, experiments were done with following arrangements within environmental chamber:

a) Introduction of different types of water in the experimental aquariums [tap water (pH 6.5) and pond water (pH 6.2) with 12 hours light: 12 hour dark condition].
b) Introduction of aquatic vegetation (such as 3 *Pistia* leaves and 5 *Hydilla* stems in the experimental aquarium).
c) Introduction of altered light (experiments were carried out in three different sets, viz. 24 hours light and 24 hours dark).

For each of the above experimental set up, a single *N. notopterus* was kept in a glass aquarium filled with 15 L of pond water and 2000 third instar *Cx. vishnui* larvae/ day were given as food and the consumption rate was noted after 24 hours. Each of the experiments was repeated thrice to reach a definite conclusion. Larvae were provided with sufficient amount of larval food.
During each of the experiment the predator was fed to satiation and then starved for 24 hours before their utilization in experiments to equalize the hunger level. The pond water was taken from the same habitat where the predator species was collected. When the experiment was carried out the range of water temperature was maintained at 29 °C to 36 °C.

2.4. Functional response analysis
The observed data were analyzed for the functional responses applying R statistical software (© The R Foundation, 2013) and MS Excel 2010. Functional response was analyzed using two steps. At first step, the shape of the functional response was determined by using the polynomial logistic regression of the proportion of prey consumed (N_a/N_0) as a function of initial prey density (N_0) (upto cubic estimate) as described by Juliano *et al*. (2001) [19].

$$\frac{N_a}{N_0} = \frac{\exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}{1 + exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}$$

where N_a is the number of prey consumed, N_0 is the initial prey density; P_0, P_1, P_2 and P_3 are the intercept, linear, quadratic and cubic coefficients, respectively, to be estimated. The sign of the coefficients P_1 and P_2 are used to determine the shape of the curve indicating the functional response. When the linear coefficient is negative $i.e. P_1 < 0$, the number of consumed prey approaches the asymptote hyperbolically as the initial prey density increases and thus describes a type II functional response. The coefficients of the logistic regression were predicted by using the function "**glm**" in R software.
In the next step, when the shape of the curve indicating functional response was found, the parameters of the type II functional response were estimated by using Holling Disc equation (Holling 1966) [20] based on the assumption of unchanging prey density over the time during the experiment:

$$N_a = \frac{aN_0T}{1 + aN_0T_h}$$

where a = the attack rate constant, T_h = handling time per prey and T = total time available (here, 24 hours). The parameters a and T_h were calculated by using Microsoft Excel 2007.

3. Results
N. notopterus was found to be a very potential predator on mosquito larvae. During observation of the predatory behavior of the fish, was found to move actively to the bottom of the aquarium and was devouring mosquito larvae when those were coming at the bottom of the aquarium for feeding. However, not all encounters led to successful grasping of the larvae as also reported earlier by Ghosh *et al*. (2011) [21].
The result of short term bioassay experiment in the laboratory revealed that the feeding rate increased with the days from the lowest of 650 larvae on the 1st day to 1039 larvae on the 7th day (Fig 1). The length and weight of the fish just after completion of the experiment were 15 cm and 19.6 gm respectively.
The effect of habitat heterogeneity and changes in the predation rate are presented in Fig 2. It was revealed that the fish consumed more larvae in presence of pond water than tap water. The predation rate was higher in presence of light than in absence of light, indicating that the fish probably utilized visual sensation during its hunting as lowest rate of consumption was noticed in complete dark condition. Aquatic vegetation reflected insignificant role in changing the rate of larval consumption. In Table 1, single factor ANOVA analysis has been presented on the effect of habitat modifications over the predation rate of *N. notopterus*. It was observed that changes in the habitations had significant effect on the rate of consumption of *Cx. vishnui* larvae as calculated table value (F=969.76) was significantly higher than the table

value ($F_{crit=}$3.11). The results of one tail paired t test showed that highest rate of consumption were noticed in pond water with 12: 12 Light: Dark condition and it is significantly different from the rate of consumption in presence of aquatic vegetation (t=4.652), in presence of 24h light (4.229) and 24 h dark (75.25); however the difference is insignificant in presence of tap water (1.245) against the table value of 2.919.
From the data it was also found that *N. notopterus* exhibited an initial sharp increase of predation rate with increase in prey density. Associated linear, intercept and quadratic coefficient values are presented in Table 2. *N. notopterus* demonstrated a type II functional response because the logistic regression estimated a significant negative linear parameter (P1<0). Figure 3 further confirmed the functional curve as Type II where proportion of mosquito larvae (prey) consumed was plotted against initial prey density given. So, the Holling Disc Equation can be applied to estimate instantaneous attack rate (a) and handling time (T_h) and the corresponding handling time was 0.15 min and attack rate was 2.95.

Table 1: Results of Single factor ANOVA analysis to establish the effect of habitat modifications on the predation rate of *Notopterus notopterus* on *Culex vishnui* larvae

Source of Variation	SS	df	MS	F	P-value	F crit
Between Habitats	3946942	5	789388.5	969.7647	3.31E-15	3.105875
Within Habitats	9768	12	814			
Total	3956710	17				

SS: Sum of square; df: degrees of freedom; MS: mean square

Table 2: Results of parameters of logistic regression analysis of the proportion of *Culex vishnui* larvae (3rd instar) consumed by *Notopterus notopterus* on increasing prey density

Parameters	Estimate	R^2 value
P_0	5145.3352	0.998
P_1	-5.4835	
P_2	0.0022	

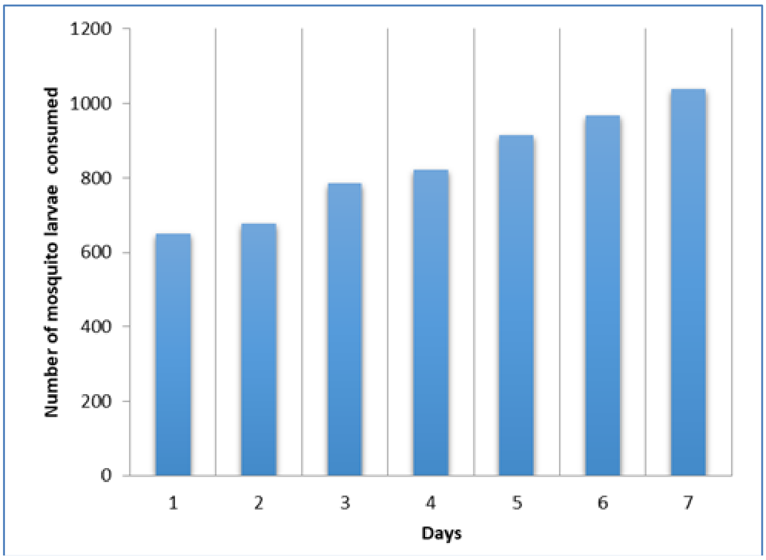

Fig 1: Change in the predation rate of *Notopterus notopterus* on *Culex vishnui* larvae in laboratory bioassay experiment

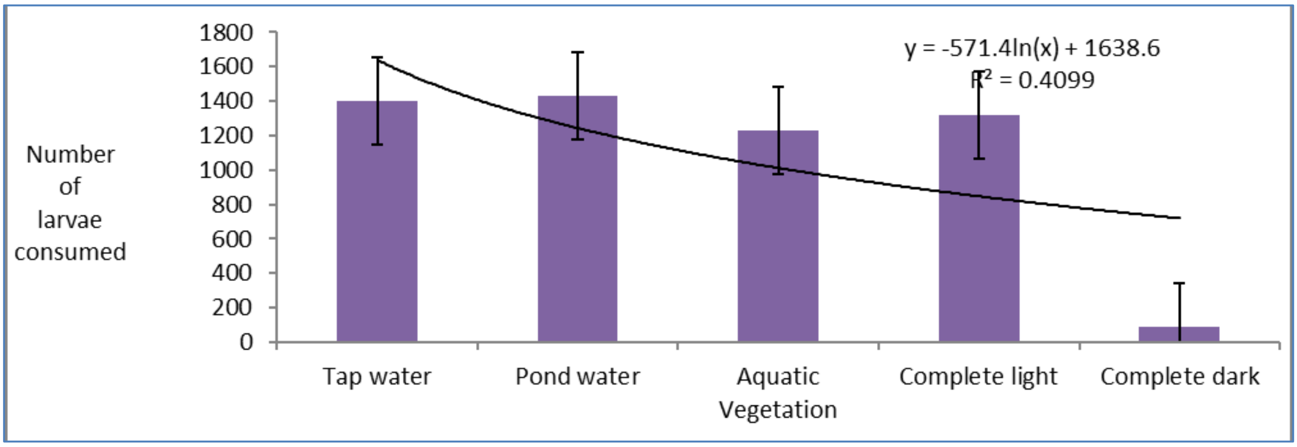

Fig 2: Changes in the rate of larval consumption of *Notopterus notopterus* in different habitat condition

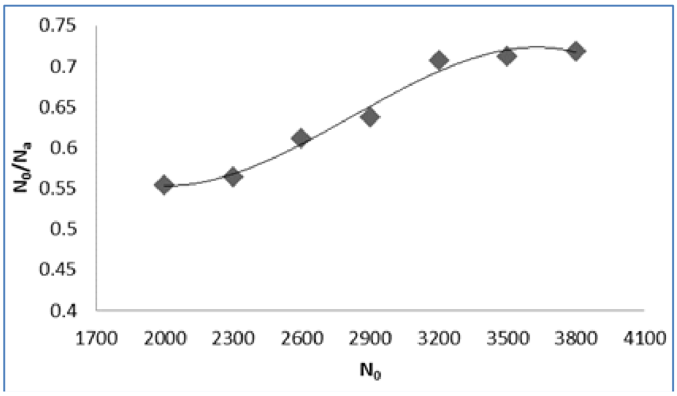

Fig 3: Functional response curve of *Notopterus notopterus* against varying prey densities

4. Discussion

Mosquito control through larvivorous fishes was practiced worldwide since pre DDT era that is from early 1900s. Recognizing its high larvivorous potentiality, the fish *Gambusia affinis* was introduced in different parts of the world from its native place Texas since 1905. Another larvivorous fish, *Poecilia reticulata* was also introduced in India during 1908 [22]. The main criteria that a fish should fulfill to qualify as a good larvivorous fish are, the fish must be hardy, can move easily through aquatic weeds, must be drought resistant, capable of flourishing in both shallow and deep water, must be a prolific breeder in the confined water also and carnivorous in habit [23].

Two main factors determine the predatory efficacy of a fish species over mosquito larvae are the stability of the fish species to the water bodies where the vector species used to breed and the ability of the fish to eat enough larvae of vector species in alternative habitat. The indigenous fish, *N. notopterus* is very well established in natural condition of Indian aquatic ecosystem, can breed in running and stagnant water in rainy season. The present study in the laboratory condition first time reported a high consumption rate with significant changes in the consumption rate due to habitat heterogeneity.

In a preliminary study Rama Rao (2014) [24] mentioned only that fry of *N. notopterus* had larvivorous capability. But present experiment revealed the larvivorous efficacy of adult *N. notopterus* in details and changes in the rate of larval consumption in different habitat conditions. Though a report on functional responses of some piscivorous fishes on other species of fishes is available [25], information regarding functional response of a fish species as a predator on mosquito larvae is scanty [10]. *N. notopterus* exhibited a Type II functional response indicating an initial increase in feeding rate with increased prey (mosquito larvae) density and then either a stable or a declining feeding rate as also found in an insect species like *Anisops sardia* [26]. Less handling time of 0.15 min of *N. notopterus* during predation of mosquito larvae indicates its high efficiency as larval control agent. Present piece of study manifested that this indigenous species of fish, *N. notopterus* can be used in mosquito control after proper semi field and field evaluations.

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