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Larvicidal efficacy of freshwater prawn *Macrobrachium lamarrei* H. Milne Edwards, 1837: a perception for mosquito bio control

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Abstract

Background: The freshwater prawn *Macrobrachium lamarrei* H. Milne Edwards, 1837 (Crustacea: Decapoda) is a common inhabitant in the wetlands including paddy fields of India. To develop and promote this predator for controlling mosquitoes, laboratory evaluation of the predation potential of *M. lamarrei* on *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae) larvae, a vector mosquito for Japanese encephalitis was made.

Methodology: A preliminary survey of different habitats was made where both prey and predators were found. Laboratory evaluation on predation potential of *M. lamarrei* in different densities and search volumes on different larval stages was made and pupal emergence of the mosquitoes was estimated.

Result: It revealed that a single adult morph of *M. lamarrei* can kill and consume 1 to 120 numbers of mosquito larvae per day depending on the prey instars, prey density and scanned volume of water. The preference of prey instars as revealed through the rate of predation remained II > III > I > IV, while the maximum amount of predation was observed at the lowest volume of water. The attack rate ranged between 0.07 L - 4.69 L and the handling time ranged between 0.21 min - 9.32 min, which differed with the volume of water in the experiments. The clearance rate (*CR*), an index of predation, ranged between 0.02 ± 0.01 to 1.8 ± 0.01 prey larvae h^{-1} $predator^{-1}$ indicating a high level of prey searching and killing efficiency.

Conclusion: The results of the present experiments effectively explored *M. lamarrei* as a potential regulatory force for controlling larval mosquito populations in natural habitats.

Keywords: *Macrobrachium lamarrei*; *Culex quinquefasciatus;* larval predation; attack rate; biological control

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1. Introduction

Mosquito-borne illnesses such as encephalitis, dengue fever, and malaria are dangerous diseases spreading quickly and can claim many live if they are not completely eradicated. More than 7×10^6 individuals worldwide are infected with mosquito-borne diseases each year, and 2×10^6 of those die [1]. In 2016, according to the Directorate of Health Service (DHS), Government of West Bengal, India, 35236 cases of malaria, 22685 cases of dengue fever, and 2004 cases of acute encephalitis syndrome (AES) were reported from West Bengal, resulting in 59, 45, and 275 deaths, respectively. Thus diseases spread by mosquitoes, has had serious human health concerns in recent years in eastern part of India [2, 3].

Besides causing biting nuisance, members of *Culex* species are the primary vectors of some human pathogen in India and worldwide, including lymphatic filariasis, Japanese encephalitis and West Nile encephalitis [4]. Vector-borne diseases including Japanese encephalitis are rapidly spreading world-wide with increasing geographic expansion. The regional dispersal of the virus is abetted by the collective effects of climate change, increased deforestation, construction of irrigation projects, change in bird migration patterns and increased movement of human and good. Since 1973, Japanese encephalitis epidemics have happened in different states of India including Andhra Pradesh, Assam, Bihar, Karnataka, Tamil Nadu, Uttar Pradesh, and West Bengal [5].

Sporadic reports of Japanese encephalitis from different districts of West Bengal reveal the disease's endemicity in this eastern region of India [6, 7]. In most of the cases, the infections are insignificant or asymptomatic but approximately 50% of patients suffering from encephalitis experience long-term brain damage, and 30% of them die [8]. Higher incidences of Japanese encephalitis were recorded during monsoon and post monsoon months due to increased prevalence of the vector mosquitoes [5, 6, 9].

Vector control remains the most successful strategy for the suppression of mosquito borne diseases, most commonly by reduction of the vector population either by environmental sanitation or by killing the vectors with various biocides. The rise of insecticide resistance soon dashed such hopes. Moreover, the application of insecticides and the environmental pressure may alter the genetic structure of vector populations in terms of insecticide resistance and susceptibility or refractoriness to infection [10]

Various means are used to control mosquitoes; among them, biological control methods, often involving predation, competition and disruption of physiological processes or a combination of these, are popular for their low ecological impact [11], reduced side effects on humans and are considered as the best alternatives to synthetic insecticides offering great promise in controlling vector populations below threshold levels [12, 13].

Although there has been an increased emphasis on studying host –pathogen dynamics within a community context, research has largely focused on a limited set of interactions [14]. The study of species interaction and community structure in lentic environments is important to determine the health of the aquatic bodies around metropolitan cities to monitor the effects of urbanization. In spite of the fact that these areas constitute important sites for subsistence fishing, little information is available about the biology and ecology of the main organisms exploited [1, 15]. Predation often has major impacts on community structure by suppressing the prey populations directly and thereby altering the trophic cascades [16]. In the suburban areas around Kolkata, the edible freshwater prawn *Macrobrachium lamarrei* H. Milne Edwards, 1837 (Crustacea: Decapoda) is dominant, acting as both predators of aquatic invertebrates as well as prey (in the larval stages) to the piscine fauna of these wetlands. Hence, it is important to know about its predatory efficacy and subsequently to quantify the degree of species overlap and utilization of space by the species.

Functional response is the basic mechanism inherent in prey-predator dynamics and describes the rate at which a predator consumes prey. Among the factors affecting functional response, attack rate and handling time are the most important [17, 18] parameters. Handling time depends on the prey type, spatial heterogeneity, prey size, prey density [19, 20] and predator feeding rate [21]. Arthropod predation also depends on several aspects of predator-prey interactions [22] including the habitat complexity [23]. Functional response can be observed, which in certain situations may be useful in monitoring patterns and strategies of attack, regulating population dynamics important to biological control [21, 24].

However, the predation potential of prawn *M. lamarrei* on mosquito larvae of *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae), has not yet been investigated. This aquatic macroinvertebrate predator was reported to coexist abundantly with other organisms, including the aquatic stages of several species of mosquito in rice fields [25-27] and wetlands [28], and have an extremely broad food niche among which mosquito larvae is the most abundantly available prey [29-31]. Generally areas colonized by aquatic vegetation are important habitats for both of these organisms (prey as well as predator), providing refuge and abundant food [32, 33]. Therefore, the present study was hypothesized that assessment of predation of this top predator would reflect upon the possible intra-guild species interactions that play an important role in organizing the structure of freshwater aquatic communities.

Hence the objectives of the present study were firstly to survey the preliminary nektonic composition to get an overview of the floral and faunal insight in different natural mosquito breeding habitats available around Kolkata, India. Secondly evaluating the predatory efficacy of *Macrobrachium lamarrei* on *Culex quinquefasciatus* larvae. Lastly estimation of predatory impact of *M. lamarrei* on *Culex* larvae.

2. Materials and methods

Survey of different natural mosquito breeding habitats

Four probable major mosquito breeding habitats were selected from the suburban areas near Kolkata, viz. old abundant water storage tanks, rice fields, temporary water pools and eutrophic ponds and a preliminary nektonic survey was conducted to get an overview of the floral and faunal composition. Plankton nets (100 μm mesh size) were used to sample the floating plants and animals in the subsurface of water. They were collected and identified up to Genus level following Chowdhury and Chowdhury [34], Khan and Ghosh [35], Ramakrishna and Dey [36].

Collection and rearing of Macrobrachium lamarrei in laboratory

Subadult, (60-69 mm; from rostrum tip to telson) stages of *Macrobrachium* identified as *Macrobrachium lamarrei* [33] were collected from wetlands in vicinity of the earlier surveyed area, acclimatized and maintained in the laboratory in low densities in 4 large aquaria of

60 × 30 × 30 cm^3 containing filtered pond water and twigs of *Vallisneria* sp., *Pistia* sp. and *Jussiaea* sp. were introduced in the rearing tanks serving as resting sites for the water bugs and to simulate natural condition. The prawns were fed ad libitum with mixed populations of larval stages of *Culex* sp. The predators were then starved for 24 h before the onset of the experiments.

Collection and rearing of Culex quinquefasciatus in laboratory

From the sewage drains of Ballygunge Science College campus, Ballygunge, Kolkata, India, mosquito larvae were collected and were brought to the laboratory. From the heterogenous population, different instars of *Cx. quinquefasciatus* larvae were separated by sieving. The III and IV instar larvae were used for experimentation. While for small sized prey, larvae were obtained by rearing the egg rafts collected from the drains. The rafts were placed within beakers containing de-chlorinated tap water. Yeast granules were provided as food after hatching of zero day-old larvae and water was changed every 24 h.

Experiment 1

It is necessary to consider the way in which the artificial environment resembles and differs from the natural system. Laboratory conditions do not completely reflect the complex interaction between predator and prey. The attack rate of predator may be altered by the availability of alternative prey [37, 38]. During experimentation, transparent circular polyethylene containers with 6 cm, 8 cm, 16 cm and 24 cm of radius and respective 12 cm, 14 cm, 14 cm and 15 cm height were taken to accommodate 0.5 L, 1.0 L, 5.0 L and 10.0 L of filtered pond water having a pH of 7.5-8.5 and temperature at 25-30 °C. These volumes reflect the representative mosquito habitats associated with the sampled water bodies. In each container different instars of *Cx. quinquefasciatus* larvae (I, II, III and IV instars) were given separately. All the instars of *Cx. quinquefasciatus* larvae were exposed to be predated at various densities, viz. 30, 60, 90, and 120 larvae $container^{-1}$. One 48 h starved *M. lamarrei* was selected to predate the larval mosquitoes of each container. Few twigs of *Vallisneria* sp. and *Jussiaea* sp. were added as refuge for both prey and predator. Experiments were done in all possible combinations considering prey densities, water volumes and larval instars in 9 replicates each, and were observed for 24 h. The containers were covered with mosquito net for preventing the predators from jumping out. To evaluate the predatory potential of *M. lamarrei*, univariate analysis of variance (ANOVA) technique [39], with transformation of the count data by square root transformation using $\sqrt{x+0.5}$ was performed.

(a) Handling time (T_h) and attack rate (a)

For evaluating behavioral pattern of predators against prey, functional response is to be calculated. It is the basic mechanism inherent in prey-predator dynamics. To estimate Type II functional response Holling equation [19] is employed based on $H_a = aHT/1 + aHT_h$. Handling time (T_h) is the time duration that a predator spends on a capturing, subduing and consuming a prey. It is one of the major parameters of functional response models along with attack rate. Attack rate (a) depicts the frequency with which a predator encounters and consumes a prey. Therefore, attack rate is the encounter rate between a predator and prey which is assumed to increase linearly with prey density considering predator is searching for food.

The Holling equation [19] can be modified to a linear form, $1/H_a = (1/a)(1/HT) + (T_h/T)$, where H_a = average number of preys killed, H = number of prey per container, T_h = handling time (in min), T = total handling time (in min) and a = attack rate (in L). The unit of attack rate is considered as 'liter' because the calculation has direct relationship with exposed volume of medium.

(b) Clearance rate (CR)

Clearance rate refers to the rate at which a predator captures, kills and consumes prey within a given space and time frame. Clearance rate (CR) is an index of predation and were calculated following Gilbert and Burns [40] with required modifications [43], using the following formula, $CR = V \ln P_e / NT$, where CR = number of prey killed volume day^{-1} predator^{-1}; P_e = number of prey killed day^{-1}; V = volume of water (in L); N = number of predators (here N = 1) ; and T = time (in h). The data obtained on CR values were subjected to two-way univariate ANOVA [39] with transformation of the count data by square root transformation using √x+0.5 was performed.

Experiment 2

Pupal emergence was also observed in presence and absence of predators. Transparent circular polyethylene containers with 24 cm of radius and 15 cm height accommodating 10.0 L of filtered pond water were taken. 1000, 0 day old, I instar larvae were kept in presence of *M. lamarrei* as predator. The number of predator varied from 1-3 along with a control devoid of predator. Three such replicates were observed at a time. The larvae were fed with Leviest® capsules each for every container during experimentation. Experiments were carried out in 25-30 °C in presence of aquatic vegetation for simulating natural conditions. Count was made every 24 h for counting the consumed larval prey and emerging pupa.

3. Results

From the preliminary survey, it was found that *Culex* sp., *Aedes* sp. and *Macrobrachium* was more or less found in all the aquatic habitats (Table 1). *Culex* being a vector for life debilitating diseases like Japanese encephalitis, Bancroftian filariasis, etc. [41] was chosen a priori as prey and *Macrobrachium* as predator species for further experiments.

Table 1: Percentage abundance of community composites in different lentic water storing bodies found in suburban areas around Kolkata.

	Species	Old abundant water storage tanks	Rice fields	Temporary water pools	Eutrophic ponds
Macraophytes	*Lemna* sp.	15.5 ± 1.59	14.17 ± 3.09	-	-
	Pistia sp.	1.86 ± 0.52	2.74 ± 0.52	7.83 ± 1.64	15.5 ± 2.29
	Jussia sp.	-	-	1.83 ± 0.31	2.17 ± 0.31
	Eichhornia sp.	-	-	-	2.5 ± 0.22
	Telenthera sp.	-	-	3.83 ± 0.54	2.33 ± 0.21
	Eloidea sp.	1.66 ± 0.52	-	-	-
	Azolla sp.	-	10.5 ± 0.96	-	-
	Wolffia sp.	-	10.67 ± 5.16	-	-
	Hydrilla sp.	-	2.48 ± 0.37	0.67 ± 0.21	-
	Myriophillium sp.	-	-	1.5 ± 0.34	-
	Paddy plants	-	13.62 ± 0.45	-	-
Arthropods	*Culex* sp. larva	127.5 ± 29.09	66.98 ± 2.92	186.83 ± 23.13	73.5 ± 2.49
(Diptera)	*Anopheles* sp. larva	-	195.83 ± 7.31	22.33 ± 5.58	-
	Mansonia sp. larva	-	-	-	1.17 ± 0.48
	Aedes sp. larva	137.5 ± 13.89	123.42 ± 3.76	163.7 ± 8.31	44.27 ± 4.73
	Armigeres sp. larva	17.5 ± 8.04	-	-	-
	Chironomous sp. larva	16.5 ± 4.54	43.67 ± 2.47	33.67 ± 6.28	1.17 ± 0.31
(Odonata)	Dragonfly nymph	-	-	-	2.83 ± 0.31
(Hemiptera)	*Anisops* sp.	-	11.83 ± 1.58	3.67 ± 0.42	8.33 ± 1.05
	Diplonychus rusticus	-	-	6.55 ± 0.26	3.5 ± 0.56
	D. annulatum	-	-	-	1.33 ± 0.49
	Ranatra sp.	-	-	-	1.67 ± 0.49
	Laccotrephes sp.	-	-	0.33 ± 0.21	0.83 ± 0.4
(Gerridae)	Water strider	0.67 ± 0.33	-	1.67 ± 0.33	-
(Acaridae)	Acari	27.17 ± 4.53	-	-	-
(Decapoda)	*Macrobrachium* sp.	17.67 ± 0.7	47.64 ± 3.89	22.48 ± 2.84	86.44 ± 8.14
Molluscs	*Gyraulus* sp.	9.17 ± 1.54	15.83 ± 1.99	18.65 ± 1.63	26.5 ± 3.33
	Lymnaea sp.	-	5.17 ± 1.97	6.33 ± 1.26	14.17 ± 1.58
	Pila sp.	-	2.67 ± 0.76	0.33 ± 0.21	1.17 ± 0.83
	Gabbia sp.	-	8.74 ± 1.97	-	20.67 ± 1.5
	Indoplanorbis sp.	-	-	-	4.33 ± 1.13
	Bellamya sp.	2.16 ± 0.56	2.83 ± 0.7	-	17.33 ± 1.52
Annelids	*Tubifex* sp.	19.33 ± 5.48	27.83 ± 5.53	39.67 ± 6.94	26.58 ± 3.87
	Branchiodrillus sp.	9.23 ± 2.44	6.17 ± 2.15	-	-
	Earthworm	-	1.5 ± 0.43	-	-

Prey consumption was significantly higher (P<0.05, df=3) in higher densities in different search volumes, except for instar IV. In 0.5 L search volume the consumption did not.significantly vary (P>0.05, df=3) with different densities of instar IV *Cx. quinquefasciatus* larva. Similarly in 1.0 L search volume, the prey consumption of *M. lamarrei* was insignificant between 30, 90 and 120 larval density of IV instar *Cx. quinquefasciatus*. In general, the consumption of all the *Culex* larval instars were recorded to be significantly high (P<0.05) in higher densities in all the volumes searched by the predator *M. lamarrei*.

Prey consumption was significantly lower (P>0.05, df=3) for instar IV in all the densities in 0.5 L, 1.0 l and 5.0 L search volumes. However, in 10.0 L search volume, it was seen that in 90 larval densities, consumption on instar I larvae along with IV instar larvae were significantly lower in comparison to other two instars of prey. In general, irrespective of densities I, II and III instar larvae were most preferred by *Macrobrachium* as a predator in all the search volumes (Fig. 1). When Duncan's test at 5% significance level was done following post hoc analysis (Table 2) it was observed that the maximum predatory efficiency of *M. lamarrei* was shown during 5.0 L search volume (55.63 ± 0.51), which decreased gradually through 1.0 L, 0.5 L and a minimum in 10.0 L (33.02 ± 0.51) search volume where prey larvae instars and prey density were not considered.

(a) Handling time (T_h) and attack rate (a)

The attack rates of *M. lamarrei* was found significantly high (χ^2=0.93, df=3, P<0.01) on IV instar of *Cx. quinquefasciatus* and lowest on II instar larvae in 0.5 L search volume (Fig. 2). At 1.0 L, minimum attack was observed on IV instars (0.07 L), while, maximum was on II instars (0.98 L) (χ^2=0.82, df=3, P<0.01). In 5.0 L search volume, the attack rate was significantly high (4.06 L) (χ^2= 0.26, df=3, P<0.01) on I instars and diminished to 1.89 L on III instar. In 10.0 L scanned water, significant (χ^2=0.2, df=3, P<0.01) variation between the larval instars were enumerated where maximum was on I instar (4.69 L) and minimum on III instar (3.1 L).

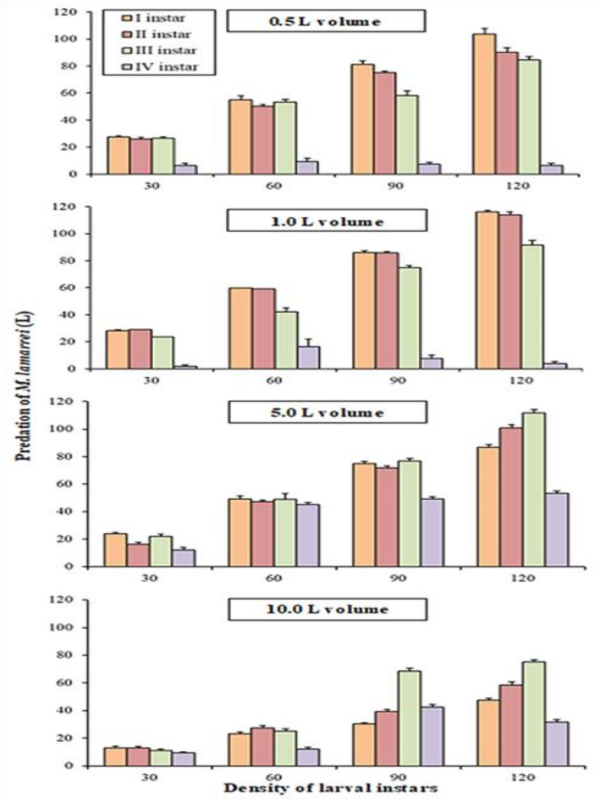

Figure 1: Consumption of different larval stages of *Cx. quinquefasciatus* by *M. lamarrei* in different prey densities and in varied search volume.

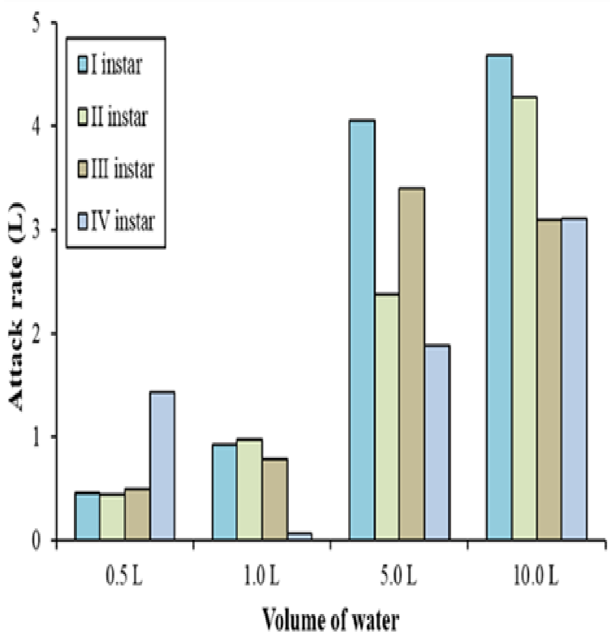

Figure 2: Attack rate (a) of *M. lamarrei* on different larval stages of *Cx. quinquefasciatus* in varied search volume.

Volume of water scanned	Consumption of *Cx. quinquefasciatus* larvae	Larval instars of prey	Consumption of *Cx. quinquefasciatus* larvae	Prey density	Consumption of *Cx. quinquefasciatus* larvae
0.5 L	47.528 ± 2.56 d	I	56.667 ± 1.058 d	30	18.111 ± 2.24 d
1.0 L	52.542 ± 4.69 c	II	56.5 ± 6.337 c	60	39.014 ± 3.482 c
5.0 L	55.632 ± 6.18 b	III	55.875 ± 2.481 b	90	57.417 ± 7.339 b
10.0 L	33.021 ± 1.39 a	IV	19.681 ± 3.228 a	120	74.181 ± 5.61 a

Table 2: Consumption (Mean ± SE) of *Cx. quinquefasciatus* larvae by M. lamarrei in different volumes of water, different prey larval instars and diverse density of larval prey by post hoc analysis N.B. Similar alphabets denote homogenous means due to Duncan's test at 5% level of significance.

The handling time of *M. lamarrei* significantly increased (χ^2=0.95 at *df*=3, *P*<0.01) from 0.37 min in I instar to 5.89 min in III instar larvae in 0.5 L search volume (Fig. 3). In 1.0 L of search volume, minimum handling time was recorded with the II instars (0.21 min), while, maximum was with IV instars (8.92 min) (χ^2=0.82 at *df*=3, *P*<0.01). The handling time (5.79 min) in III instars larvae significantly diminished (χ^2=0.93 at *df*=3, *P*<0.01) to 0.45 min (in I instar) in 5.0 L search volume. Significant (χ^2=0.03, *df*=3, *P*<0.01) variation between the larval instars were observed in 10.0 L search volume having maximum in III instar (2.36 min) and minimum in I instar (9.32 min).

(a) Clearance rate (CR)

The clearance rate (*CR*) of the crustacean decapod depended on the volume of medium searched by the predator (Table 3), and revealed a significant variation (*F*=25532.05, *df*=3, *P*<0.01). Again, when consumption of larval instars (*F*=201.95, *P*<0.01, *df*=3) and density of the prey (*F*=926.97, *P*<0.01, *df*=3) were analyzed, it also exhibited significantly. Duncan's test at 5% significance level followed by post hoc analysis showed minimum clearance rate of *M. lamarrei* during 10.0 L search volume (1.37 ± 0.004) to a maximum in 0.5 L search volume (0.07 ± 0.004) where prey larval instars and their densities were not considered (Table 4). Irrespective of volume searched by predator and prey densities, when *CR* of larval instars were taken into account, it was maximum on III instar (0.64 ± 0.004) and minimum on IV instar (0.52 ± 0.004). Similar trends were followed by II and I instar larvae. Considering prey densities, a decrease in *CR* was observed from 0.71 ± 0.004 at '120 prey density' to a minimum of 0.44 ± 0.004 at '30 prey density'.

Role of predator on prey death

The death rate of *Cx. quinquefasciatus* larvae was significantly (*P*<0.05) higher in absence of predators during early days of larvaldevelopment (day 1, 2, 4, 5, 7, 9) in comparison to the later days (day 10, 11, 12, 13, 14). Similar trend was followed in presence of predator/s. However, in presence of predators the death rate is significantly higher even in 1st three days in comparison to the next consequent days (Table 5).

On day 1 the death rate was significantly high in presence of 2 and 3 predators. On day 2, the maximum death (P<0.05) was observed in presence of 2 predators followed by 3 predators. However, on day 3 the maximum death was noticed in presence of single predator. No significant differences in death rate were observed from day 4 to day 13 (Table 5).

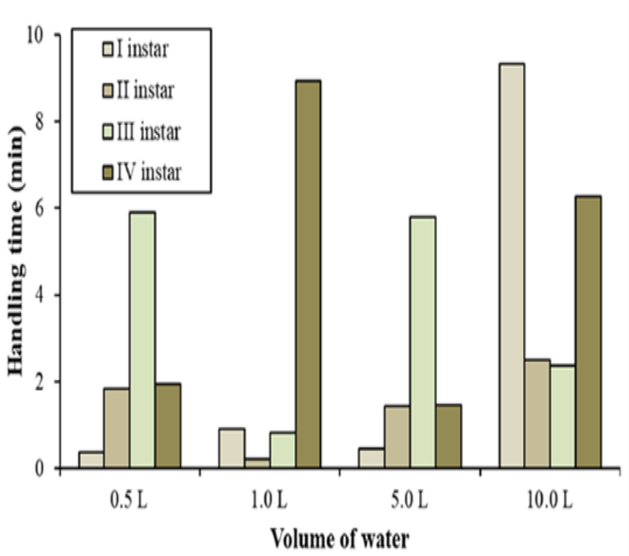

Figure 3. Handling time (Th) of *M. lamarrei* on different larval stages of *Cx. quinquefasciatus* in varied search volume.

Pupal emergence in absence and presence of predators

Pupal emergence was significantly higher after day 10 and was found to be maximum on day 14 in absence of predator. When a single predator was incorporated in the experiment, the emergence of pupa was observed to be highest on day 13. When 2 predators were present, the pupal emergence maximized on day 11. In presence of 3 predators maximal emergence of pupa was day 10.

Pupal emergences were insignificant for initials days in absence or presence of predators. Even after day 5, pupal emergence due to presence of predators was not significant up to day 9. On day 10 the pupal emergence was significantly high after incorporating 2 or 3 predators (Table 6).

4. Discussion

Macrobrachium lamarrei is one of the most common and widely distributed freshwater macrocrustaceans which is important in littoral communities of shallow lakes and streams of the floodplain of the Indian subcontinent, as well as in West Bengal. The relative abundance of mosquito immatures in aquatic water bodies probably reflects more encounter of the freshwater prawn with them, which are relatively large and sluggishly moving prey. *M. lamarrei* is omnivorous [42], but with an important carnivorous component. It prefers mainly dipteran larvae, oligochaetes and detritus [42].

From the results, it is evident that the decapod macrocrustacean consumed effectively upon mosquito larvae (Fig. 4) at its different stages, and the rate varied significantly between them. Simultaneously, the prey consumption potential varied significantly between various prey densities and different search volumes.

Density	Instars of *Cx. quinquefas*	Volume of water (L)			
		0.5	1.0	5.0	10.0
30	I	0.06 - 0.07 0.07 ± 0.001	0.13 - 0.14 0.14 ± 0.001	0.61 - 0.69 0.66 ± 0.01	0.92 - 1.25 1.05 ± 0.04
	II	0.06 - 0.07 0.07 ± 0.001	0.14 - 0.14 0.14 ± 0.0004	0.5 - 0.64 0.57 ± 0.02	0.92 - 1.2 1.06 ± 0.03
	III	0.06 - 0.07 0.07 ± 0.001	0.13 - 0.14 0.13 ± 0.001	0.55 - 0.71 0.64 ± 0.02	0.81 - 1.16 0.98 ± 0.04
	IV	0.01 - 0.06 0.03 ± 0.01	0 - 0.08 0.02 ± 0.01	0.43 - 0.61 0.51 ± 0.03	0.81 - 1.07 0.93 ± 0.03
60	I	0.07 - 0.09 0.08 ± 0.001	0.17 - 0.17 0.17 ± 0.0001	0.78 - 0.85 0.81 ± 0.01	1.2 - 1.42 1.3 ± 0.03
	II	0.08 - 0.08 0.08 ± 0.001	0.17 - 0.17 0.17 ± 0.0002	0.78 - 0.83 0.8 ± 0.01	1.23 - 1.47 1.37 ± 0.03
	III	0.08 - 0.09 0.08 ± 0.001	0.14 - 0.16 0.16 ± 0.003	0.58 - 0.84 0.8 ± 0.03	1.27 - 1.43 1.34 ± 0.02
	IV	0.03 - 0.07 0.04 ± 0.004	0 - 0.16 0.08 ± 0.02	0.76 - 0.82 0.79 ± 0.01	0.81 - 1.18 1.03 ± 0.04
90	I	0.09 - 0.09 0.09 ± 0.001	0.18 - 0.19 0.19 ± 0.001	0.88 - 0.92 0.9 ± 0.004	1.36 - 1.48 1.42 ± 0.01
	II	0.09 - 0.09 0.09 ± 0.0003	0.18 - 0.19 0.19 ± 0.001	0.88 - 0.92 0.89 ± 0.004	1.43 - 1.59 1.53 ± 0.02
	III	0.08 - 0.09 0.08 ± 0.001	0.18 - 0.18 0.18 ± 0.001	0.88 - 0.92 0.9 ± 0.01	1.7 - 1.81 1.76 ± 0.01
	IV	0.02 - 0.05 0.04 ± 0.004	0.03 - 0.12 0.07 ± 0.01	0.77 - 0.84 0.81 ± 0.01	1.29 - 1.51 1.43 ± 0.03
120	I	0.09 - 0.1 0.1 ± 0.001	0.2 - 0.2 0.2 ± 0.0003	0.92 - 0.96 0.93 ± 0.004	1.54 - 1.65 1.61 ± 0.01
	II	0.09 - 0.1 0.09 ± 0.001	0.19 - 0.2 0.2 ± 0.001	0.94 - 0.98 0.96 ±0.01	1.63 - 1.75 1.69 ± 0.02
	III	0.09 - 0.1 0.09 ± 0.001	0.18 - 0.2 0.19 ± 0.002	0.96 - 0.1 0.98 ± 0.01	1.76 - 1.84 1.8 ± 0.01
	IV	0.01 - 0.06 0.03 ± 0.01	0 - 0.12 0.04 ± 0.01	0.81 - 0.86 0.83 ± 0.01	1.51 - 1.64 1.56 ± 0.02

Table 3: Clearance rate (volume day^{-1} predator^{-1}) (Range, Mean ± SE) by *M. lamarrei*, predating upon different larval instars of *Cx. quinquefasciatus* at different prey densities.

These results reflect their capability to induce stable prey mortality by high predatory potential. The predatory impact, reflecting the prey killing capability of *M. lamarrei* is expectedly more owing to its size and energy requirements. Also, variation in consumption rate by the freshwater prawn between the larval instars can be attributed towards prey developmental characteristics because certain general rules guide the pattern of arthropod predation related to prey size, prey density, search volume and other factors pertaining to the biology of predators [22, 32, 43]. When the predation rate of *M. lamarrei* is considered in respect to the prey densities, the rate of consumption varied, reflecting its ability to kill more preys. This proportionate killing reflects its higher ability towards target prey termination.

Considering these freshwater crustaceans as biological resources to control mosquito population, the present study ascertains their competence as biological control agents. Several other crustacean including *Ceriodaphnia* sp., *Daphnia* sp., *Triops longicaudatus*, *T. newberryi* (Notostraca: Triopsidae), *Macrocyclops albidus*, *Megacyclops viridis*, *Mesocyclops obsoletus, Meso. aspericornis*, *Meso. leukartipilosa*, *Meso. thermocyclopoides* (Crustacea: Copepoda), *Caridina nilotica brachydactyls*, *C. typhus*, *Macrobrachium* *borellii*, *Palaemonetes argentinus* and *Varuna litterata* (Crustacea: Decapoda) [38, 44-46] have been identified as potential predators of mosquito larvae.

The clearance rate was considered as an overall indicator of prey-predator interaction, including the space available, prey size and predation in unit time. This indicator was used for comparing the efficacy of the predator. From clearance rate (*CR*), the ability of the predator to search, capture and predate within a specific space and time can be estimated. When the predators were present within a larger surface area, the predation rate decreased, which is similar to natural situations. *CR* varied significantly between prey densities and larval instars. Thus, the predation strategy of *M. lamarrei* is different. Ambush predation is more frequent because of more availability of prey in the vegetated area near the banks of freshwater bodies. Moreover search for prey at periphery and short striking range makes prey capture more acute than cruise predators, moving continuously and scanning for prey. Ambush predation is more frequent because restoration of energy changes the feeding which depends on prey density, search time, volume of water scanned and size of the prey.

Many authors revealed that, the species composition, structural complexity and other environmental features of a habitat influence the abundance and function of aquatic predators of mosquito immature [23, 47-50]. From the viewpoint of biological control, the freshwater prawn should have a wide range of adaptability by predating the target mosquito larvae, which strengthens the idea of utilizing natural enemies for controlling vector mosquitoes, an alternative option to chemical pesticides, which adversely affects the biodiversity of the system. Here, prey density, search volume and prey preference played a major role in population regulation of target mosquito species *Cx. quinquefasciatus*. Considering *M. lamarrei* as biological resources to combat the mosquito population, the present study ascertains its competence as biological control agents. Moreover this macrocrustaceans are prolific breeders [42] and have very high larval feeding efficacy, they can be effectively used for control of various mosquito species of the wetlands.

M. lamarrei are commonly found among human habitations. They hatch abundantly in abundant water storage tanks, temporary water pools, eutrophic ponds and rice fields. Since *Macrobrachium lamarrei* lays eggs twice a year, the major peak being on the month of June with the start of the rainy season and the minor peak during December with a high fecundity rate (69-143 eggs per female) [51, 52], and *Culex* mosquitoes breeding all throughout the year [53], the interaction between freshwater prawn and mosquitoes occurs as soon as the mosquitoes start breeding. Each year in India, two peaks of Japanese encephalitis occur during premonsoon and postmonsoon months [54] and these two-monsoon season are in fact also the breeding seasons of *Macrobrachium lamarrei*. Since *Culex* has a preference for blood meal, and live in close vicinity to human dwellings [55, 56], the role of *M. lamarrei* is extremely important in reducing the populations of vectors mosquitoes [57, 58]. Thus, results of this study suggested that predation reduce mosquito larval density and hence contributes to reduced incidence of mosquito-borne diseases like Japanese encephalitis.

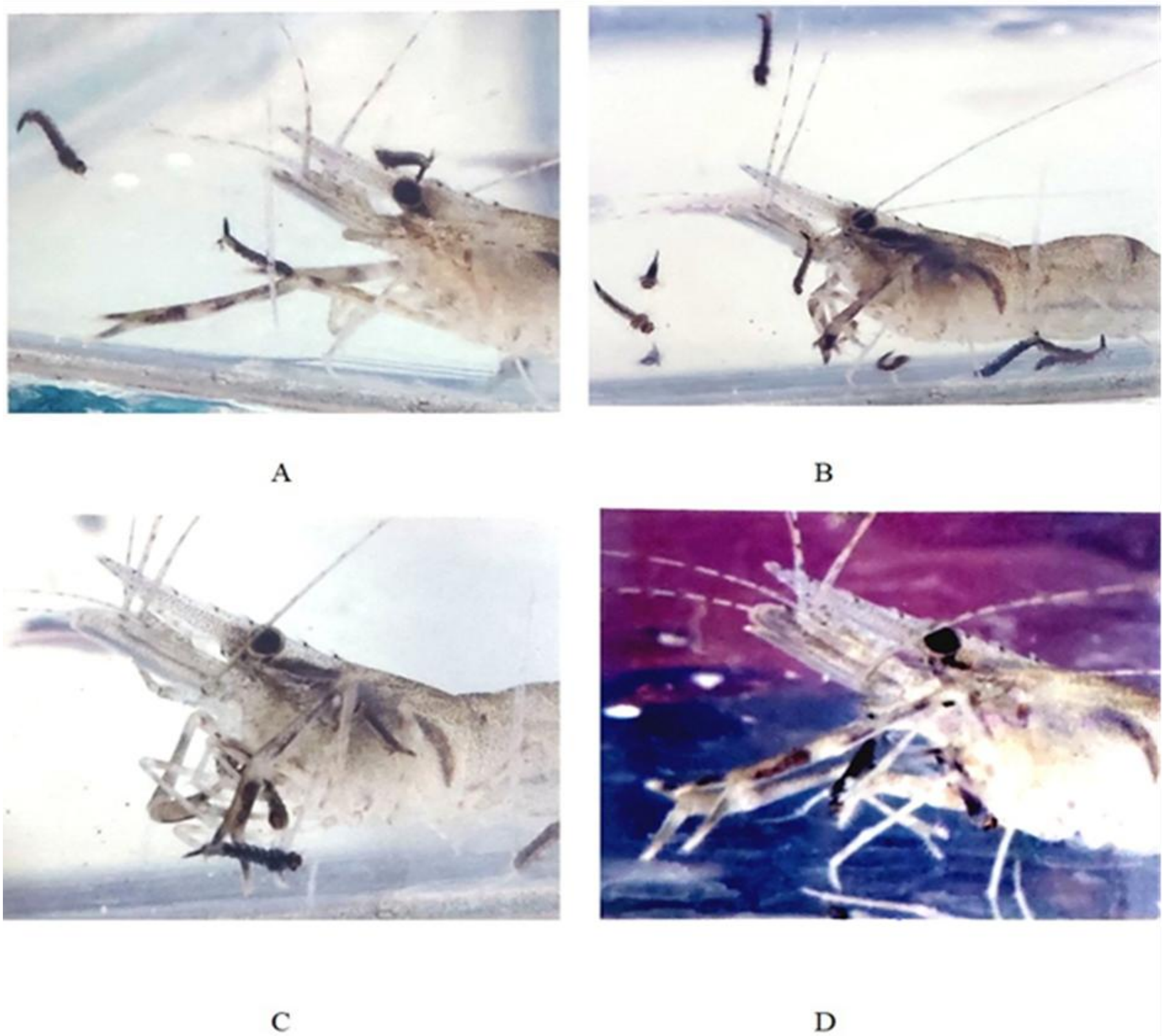

Figure 4. Sequential events (A – D) of subduing and consuming *Cx. quinquefasciatus* larva by *M. lamarrei.*

Volume of water scanned	Clearance rate on *Cx. quinquefasciatus* larvae	Larval instars of prey	Clearance rate on *Cx. quinquefasciatus* larvae	Prey density	Clearance rate on *Cx. quinquefasciatus* larvae
0.5 L	0.072 ± 0.004 d	I	0.607 ± 0.033 c	30	0.442 ± 0.004 d
1.0 L	0.141 ± 0.003 c	II	0.619 ± 0.076 b	60	0.57 ± 0.007 c
5.0 L	0.799 ± 0.043 b	III	0.637 ± 0.018 a	90	0.66 ± 0.038 b
10.0 L	1.365 ± 0.001 a	IV	0.515 ± 0.006 d	120	0.706 ± 0.002 a

Table 4: Clearance rate (Mean ± SE) by *M. lamarrei* depending separately on volume of water scanned, prey larval instars and diverse density of larval prey by post hoc analysis. N.B. Similar alphabets denote homogenous means due to Duncan's test at 5% level of significance.

Days	Predator 0	Predator 1	Predator 2	Predator 3
0	0	0	0	0
1	$76^{cda4} \pm 17.16$	$98.33^{d1e1a4} \pm 7.45$	$168.33^{c2a5} \pm 17.89$	$160^{e3a5} \pm 7.64$
2	$71.33^{bcdb4} \pm 7.84$	$89.33^{d1e1b4} \pm 13.42$	$323^{d2b6} \pm 24.27$	$186.33^{e3b5} \pm 12.71$
3	$30.33^{abcc4} \pm 17.33$	$265.33^{f1c6} \pm 15.96$	$188.33^{c2c5} \pm 3.71$	$225.66^{f3c5c6} \pm 13.86$
4	$69.33^{bcdd4} \pm 25.98$	$65.66^{c1d1d4} \pm 14.44$	$41.33^{a2b2d4} \pm 3.53$	$70^{c3d3d4} \pm 26.58$
5	$101.33^{de4e5} \pm 21.93$	$108.33^{e1e5} \pm 24.5$	$43.33^{b2e4} \pm 7.88$	$97.33^{d3e4e5} \pm 15.07$
6	$43^{abcf4} \pm 21.79$	$47.33^{b1c1f4} \pm 20.87$	$39.66^{a2b2f4} \pm 10.65$	$57.33^{c3f4} \pm 1.45$
7	$80.33^{cdg4} \pm 17.29$	$49^{b1c1g4} \pm 4.04$	$43.66^{b2g4} \pm 2.96$	$40.66^{b3c3g4} \pm 16.68$
8	$27.66^{abch4} \pm 14.11$	$36^{a1b1c1h4} \pm 11.02$	$17.33^{a2b2h4} \pm 8.11$	$39.66^{b3c3h4} \pm 18.41$
9	$80.66^{cdi4} \pm 29.01$	$26.33^{a1b1c1i4} \pm 11.84$	$5.66^{a2b2i4} \pm 3.05$	$10.66^{a3b3i4} \pm 3.53$
10	$43.66^{abcj4} \pm 13.54$	$10^{a1b1j4} \pm 4.16$	$26^{a2b2j4} \pm 14.73$	$10.66^{a3b3j4} \pm 6.67$
11	$25.66^{abck4} \pm 13.97$	$23.66^{a1b1k4} \pm 12.35$	$2^{a2k4} \pm 0.58$	-
12	$18.33^{abl4} \pm 11.92$	$9.33^{a1b1l4} \pm 3.18$	-	-
13	$18.33^{abm4} \pm 3.28$	$21.66^{a1b1m4} \pm 3.18$	-	-
14	$4^{an5} \pm 1.73$	-	-	-

Table 5: Rate of *Cx. quinquefasciatus* larval death in absence and presence of predators. N.B. Different letters with number in superscript indicate significant difference in mean (P<0.05).

Here, an insight on the role of decapod crustacean in reducing mosquito populations through destruction of mosquito larvae. Since *M. lamarrei* having different breeding sites can predate on mosquito larvae, when environmental management policy decisions are made, such as application of pesticides or introduction of predatory fish, the role of these microcrustaceans for controlling the *Culex* mosquito larvae should be considered.

5. Conclusion

In conclusion, this study sheds light on the significant role of the freshwater prawn *Macrobrachium lamarrei* as a potential biological control agent for mosquito-borne diseases, particularly focusing on the reduction of Culex quinquefasciatus larvae, a vector for diseases like Japanese encephalitis. The results demonstrate M. lamarrei's efficacy in predating mosquito larvae at different stages, highlighting its potential as a natural enemy for controlling vector mosquitoes. This study contributes valuable insights into the ecological interactions in aquatic environments, providing a basis for developing sustainable strategies in the ongoing battle against mosquito-borne illnesses.

Days	Predator 0	Predator 1	Predator 2	Predator 3
0	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	$0.67^{a2a4} \pm 0.67$	0
6	0	0	$16.33^{a2b2a5} \pm 11.29$	$14^{a3a5} \pm 5.86$
7	$1.67^{aa6} \pm 1.67$	$34^{b1a6} \pm 22.48$	$44.33^{b2c2a6} \pm 22.06$	$44.33^{b3a6} \pm 8.01$
8	$27.33^{aa7} \pm 9.06$	$59.67^{c1a7} \pm 6.69$	$66^{c2a7} \pm 28.88$	$62.33^{c3a7} \pm 1.86$
9	$121.67^{ba8} \pm 49.51$	$83.33^{d1a8} \pm 2.33$	$72.67^{c2d2a8} \pm 13.59$	$87^{d3a8} \pm 10.08$
10	$191^{bcb9} \pm 48.01$	$128.33^{a9b9} \pm 1.45$	$72.33^{c2d2a9} \pm 1.86$	$101.67^{a9} \pm 10.37$
11	$256.67^{cdc10} \pm 42.27$	$136.67^{b10} \pm 3.53$	$101.33^{d2b10} \pm 4.81$	-
12	$277^{dc11} \pm 41.24$	$143.67^{b11} \pm 6.12$	-	-
13	$292.33^{dc12} \pm 44.76$	$1496.7^{b12} \pm 10.17$	-	-
14	$310^{da13} \pm 38.03$	-	-	-

Table 6: Pupal emergence of *Cx. quinquefasciatus* in absence and presence of predators. N.B. Different letters with number in superscript indicate significant difference in mean (P<0.05).

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Conflict of interest

Author declares there are no conflicts of interest.

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