



FUNCTIONAL RESPONSE OF *Diplonychus indicus* VENK. & RAO (HEMIPTERA: BELOSTOMATIDAE) AGAINST *Aedes aegypti* LINNAEUS AND *Aedes albopictus* SKUSE (DIPTERA: CULICIDAE)

GRACE MARIN¹, ASKILLA PEARLIN¹, PAULRAJ BLESSY¹,
DEVASANTHAKUMAR GINCY¹, SUBRAMANIAN ARIVOLI²
AND SAMUEL TENNYSON^{3*}

¹Department of Zoology, Scott Christian College, Nagercoil 629 003, Tamil Nadu, India.

²Department of Zoology, Thiruvalluvar University, Vellore 632 115, Tamil Nadu, India.

³Department of Zoology, Madras Christian College, Chennai 600 059, Tamil Nadu, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors designed the study and wrote the protocol. Authors GM, AP, PB and DG managed the analyses of the study. Authors GM, SA and ST managed the literature searches and wrote the first draft of the manuscript. Author ST performed the statistical analysis and also edited the final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The predatory efficiency of the hemipteran water bug *Diplonychus indicus* was evaluated for its functional response against its prey *Aedes aegypti* and *Aedes albopictus* larvae for 72 hours with varied prey densities. The number of prey killed increased with increasing prey density from 1 prey per single predator to 32 prey per single predator. The attack rate increased with the increase of prey density level, and was at its peak at 16 prey density (22.30) and lowest at 4 prey density (3.77) for *Aedes aegypti*; and for *Aedes albopictus* it was at one prey density (7.70) and the lowest at the maximum prey density level (3.44). *Diplonychus indicus* fed on *Aedes aegypti* and *Aedes albopictus* larvae took 0.0193 days (27.79 minutes) and 0.0108 days (15.55 minutes) handling time respectively. The predator killed maximum number of 140.22 *Aedes aegypti* and 151.07 *Aedes albopictus* larvae after 72 hours, when the prey density level was 32. Regression analysis of prey density against number of prey attacked for 72 hours observation yielded a significantly positive slope for both *Aedes aegypti* and *Aedes albopictus*. The predation experiment revealed that the functional response analysis of *Diplonychus indicus* exhibited a Type II functional response. Several factors affect the functional response of a predator's

*Corresponding author: Email: samtennyson@gmail.com;

search mode, especially strike and foraging success, which are complex and can be understood only by looking at each component separately.

Keywords: *Diplonychus indicus*; *Aedes aegypti*; *Aedes albopictus*; predator-prey; functional response.

1. INTRODUCTION

Since the early development of predator-prey theory, ecologists have recognized the theoretical importance of understanding details of a predator's feeding rate. Theoretical work has demonstrated that the mathematical form of the feeding rate can influence the distribution of predators through space, the stability of enriched predator-prey systems. A predator's per capita feeding rate on prey, or its functional response, provides a foundation for predator-prey theory. Holling's prey dependent functional response, a model that is a function of prey abundance only, has served as the basis for predator-prey theory [1,2]. The fundamentals in predator-prey relationship are to evaluate the functional response of a predator which reflects on the function relating the number of prey eaten per unit time by a single average predator to the size of the prey population [3]. It is an extremely complex system where many assumptions and predictions may or may not hold well unless one nullifies all such interference factors in the predator's performance.

Understanding the relationship between predator and prey is a central goal in ecology, and one significant component of the predator-prey relationship is the predator's rate of feeding upon prey. The feeding rate describes the transfer of biomass between trophic levels, and completely describes the dynamic coupling between predator and prey abundance. Studies of predator-prey connotations relating mosquito larvae have been reported [4]. Among aquatic insects, Belostomatidae, Gerridae and Nepidae are the most significant families of predaceous hemipteran bugs [5-17]. *Diplonychus indicus* a tropical water bug, cosmopolitan in distribution, is a highly efficient predator on both Anophelinae and Culicinae larvae, and its predatory behaviour is highly versatile [17-19]. Studies have been conducted on the functional response of aquatic predators [13,20-22], since they help to obtain vital information, and could be helpful to study inter basic mechanisms of prey predator relationships. Further, functional response of aquatic predators as biocontrol mosquito agents can help to obtain significant information to make decision in mosquito management programs. Therefore, in the present study, the functional response of *Diplonychus indicus* was investigated on the larvae of *Aedes aegypti* and *Aedes albopictus* as the prey.

2. MATERIALS AND METHODS

2.1 Culture of *Diplonychus indicus*

Adults of *Diplonychus indicus* were collected from a pond in Peruvilai, near Parvathipuram, Nagercoil, Kanyakumari, Tamil Nadu, India using an insect net having 200 µm mesh size. They were then brought to the laboratory and maintained in glass aquaria (30×20×20 inches) containing pond water (10L). Few specimens of *Hydrilla* species and gravels were placed inside the aquarium to invigorate natural conditions. The bugs were acclimatized for seven days before the commencement of the experiment in the laboratory with *Aedes* mosquito larvae as food.

2.2 Culture of *Aedes aegypti* and *Aedes albopictus*

Cyclic generations of *Aedes aegypti* and *Aedes albopictus* were maintained separately in two feet mosquito cages with a mean room temperature of 27±2°C and a relative humidity of 70-80% inside an insectary and the adults were fed on 10% glucose solution in water. Ovitrap were placed inside the mosquito cages for the female mosquitoes to oviposit eggs and the laid eggs were then transferred to the larval rearing chamber and were maintained in enamel larval trays. The larvae were fed with dog biscuits and yeast in the ratio 3:1. The larvae on transforming to pupae were transferred to plastic bowls kept inside another mosquito cage for adult emergence. The third instar larval phase of these vector mosquitoes were utilized as prey for the present study.

2.3 Functional Response

To a single *Diplonychus indicus* adult, third instar larvae of *Aedes aegypti* and *Aedes albopictus* were offered separately at densities of 2, 4, 8, 16 and 32 per 250 mL of a glass trough and allowed to predate for a period of 72 hours to determine the rate of predation and the functional response. After every hour, the respective prey density was replaced for each prey density [21]. Holling's [1,2] equation comprising of various parameters was adopted to describe the functional response of *Diplonychus indicus* on *Aedes aegypti* and *Aedes albopictus*. Linear regression was used to establish the relationship between the prey density and the number of prey consumed by the

predator, and between observed attack ratio and predicted attack ratio.

Preceding the initiation of experiment, the predators were fed to satiation and then starved for 24 hours to equalize the hunger level approximately. Six replicates were performed for each experimental design and the study period was 72 hours. Controls without predators were set with equal number of replicates as those of the test.

3. RESULTS

3.1 Number of Prey Killed

Prey killed (y) by *Diplonychus indicus* at different prey densities of *Aedes aegypti* and *Aedes albopictus* are presented in Table 1. The number of prey killed by the predator progressively increased with the increase in prey density level from one prey per single predator to 32 prey per single predator, and reached its peak at 32 prey per single predator density level (Fig. 1). The predator killed maximum number of 140.22 *Aedes aegypti* and 151.07 *Aedes albopictus* larvae after 72 hours, when the prey density level was 32. Regression analysis of prey density (x) against number of prey attacked (y) for 72 hours observation yielded a significantly positive slope for both *Aedes aegypti* ($y = 4.24x + 26.21$) and *Aedes albopictus* ($y = 4.43x + 31.82$).

3.2 Attack Ratio

The number of prey killed or consumed (y) by predator in a given time did not differ significantly from the calculated (y) based on Holling's equation, for both prey species. The highest attack ratio (y/x) was observed at the density of one prey per single predator and the lowest was at 32 prey density level,

and the attack ratio decreased with increased prey density for both prey species.

3.3 Attack Rate (a) and Handling Time (b)

The attack rate (a) increased with the increase of prey density level. For *Aedes aegypti*, the peak was at 16 prey density (22.30) and lowest at 4 prey density (3.77); and for *Aedes albopictus* it was at one prey density (7.70) and 32 density level (3.44) respectively (Fig. 1). The time taken by the predator to feed the captured prey (b) was estimated as feeding time. *Diplonychus indicus* fed on *Aedes aegypti* and *Aedes albopictus* larvae took 0.0193 days (27.79 minutes) and 0.0108 days (15.55 minutes) handling time per prey respectively. The maximum predation (k) restricted to higher prey density levels, at 140.22 and 151.07 for *Aedes aegypti* and *Aedes albopictus* respectively at 32 prey per single predator density. The predicted number of prey killed (y) was more or less similar to the observed number of prey killed at various densities for both prey species. The searching time (T_s) decreased with increased prey density. At prey densities below 32 prey per single predator density, the predator spent more time for searching the prey. The searching time (T_s), the attack ratio (y/x), and the attack rate (a) decreased with increasing prey density.

4. DISCUSSION

Predation advances through successive escalating stages from initial detection of prey by predator, attack, capture and ingestion [23]. To understand the relationship between the consumption rate of a predator and its prey density, the number of prey (food) items consumed per time unit must be related to food abundance through a functional response [24]. In the prey predator contest, a significant factor

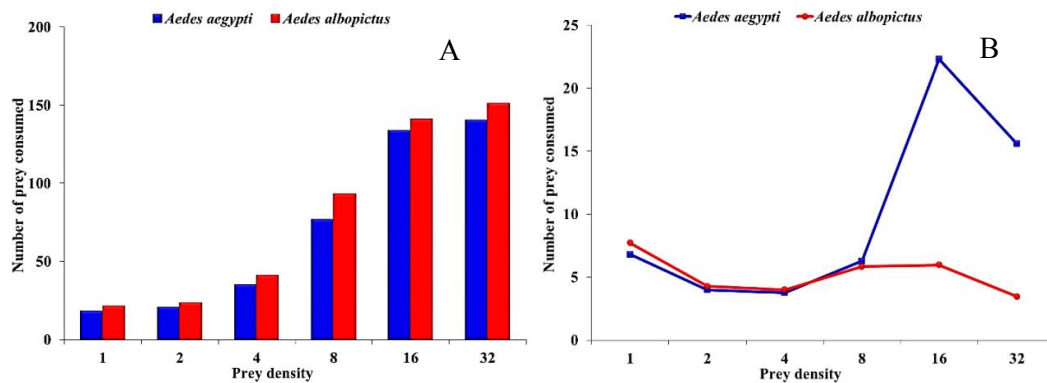


Fig. 1. Functional response (A) and attack rate (B) of *Diplonychus indicus* against *Aedes* larvae

Table 1. Cumulative functional response of *Diplonychus indicus* against *Aedes* larvae

x	y	k	b	by	Ts	y/x	k/Tt	a	Holling's equation $y' = a(Tt-by) x$	y'
<i>Aedes aegypti</i>										
1	18.03	140.22	0.01	0.34	2.65	18.03	46.74	6.79	$y' = 6.79(3-0.34)1$	18.03
2	20.74			0.40	2.59	10.37		3.98	$y' = 3.98(3-0.40)2$	20.74
4	35.09			0.67	2.32	8.77		3.77	$y' = 3.77(3-0.67)4$	35.09
8	76.57			1.47	1.52	9.57		6.28	$y' = 6.28(3-1.47)8$	76.57
16	133.81			2.58	0.41	8.36		22.30	$y' = 22.30(3-2.58)16$	133.81
32	140.22			2.70	0.29	4.38		15.58	$y' = 15.58(3-2.70)32$	140.22
<i>Aedes albopictus</i>										
1	21.33	151.07	0.01	0.23	2.76	21.33	50.36	7.70	$y' = 7.70(3-0.23)1$	21.33
2	23.48			0.25	2.74	11.74		4.27	$y' = 4.27(3-0.25)2$	23.48
4	40.82			0.44	2.55	10.20		3.98	$y' = 3.98(3-0.44)4$	40.82
8	93.04			1.00	1.99	11.63		5.82	$y' = 5.82(3-0.00)8$	93.04
16	140.86			1.52	1.47	8.80		5.95	$y' = 5.95(3-1.52)16$	140.86
32	151.07			1.63	1.36	4.72		3.44	$y' = 3.44(3-1.63)32$	151.07

x: prey density; y: total number of prey killed in a given period of time; Tt: total time when prey was exposed to predator; Ts: time spent by predator in searching the prey; a: attack rate per unit of searching time; b: time taken for handling each prey by predator; k: maximum predation

is the strike time of predator and the startle time of prey [25]. In prey predator interaction in which both are mobile, the satiation time involves two important aspects, viz., predation and consumption (handling time). According to Holling [1,2] and Hassell [26], the functional response depends on two parameters, the attack rate that represents the rate of successful attack (searching efficiency of the predator) and the handling time that is the time required for a predator individual to handle an individual prey (pursuing, subduing, eating and digesting).

Diplonychus indicus documented as biological agents for the management of larval mosquitoes, feed by piercing the prey with their rostrum by injecting digestive juices and sucking the liquid contents from the larvae. The study on the influence of prey density on the predatory efficiency of *Diplonychus indicus* revealed that the number of prey killed by *Diplonychus indicus* varied owing to functional response. Sivagnaname [27] reported that the number or prey killed by *Diplonychus indicus* increased with increasing prey density and reached a plateau at and beyond the density of 80, thereby showing optimum prey density for its predation. Further, the predatory efficiency of *Diplonychus indicus* was high at various prey densities especially for species of *Aedes* mosquitoes. Similar trend was observed in the present study too, wherein, when the prey density increased, the number of prey killed by a single predator also increased. Marin [12] reported that when *Diplonychus indicus* was exposed to varying prey densities of all instars of *Culex* larvae, the rate of predation was higher, and it was also found that the predator came to

the level of satiation only after consuming a large number of prey (10-56 larvae per hour).

Predators react to changes in density of prey. Venkatesan et al. [6] reported that predation is highly influenced by the density of the prey to which the predator gets exposed. The response of insect predators to changes in prey density varies. When the individual predator attacks more, prey density decreases and it is referred to as functional response. This aspect demands a suitable fixation of prey in estimating the efficiency of the predator. In some predators, these two parameters show a positive correlation, while in others they show a negative correlation. 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 [28]. The functional response can be studied by evaluating the parameters, viz., 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.

The attack ratio was higher in one prey density per single predator, and it gradually decreased when the prey level/density increased. The higher attack rate at lower prey densities might be due to the lesser time

required by the predator to find each prey. However, at higher prey densities, the predator spent more time for non-searching activities, which in turn caused a perceptive decline in the attack rate until hunger was stabilized. The satiated ones would not search for another prey and the attack rate decreased with increasing prey density. The study also provided a preliminary idea about the change in the predation efficiency with change in the larval density and search area from established regression equation. Holling [1,2] stated that 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.

The searching and feeding behaviour of aquatic predators generally change as population density increases. This behaviour is referred as functional response. It explains the change in the number of prey consumed per unit time in relation to prey density. Holling's [29] functional response model predicts that when the prey density remains constant, the rate of successful search and encounter rate, and inter catch interval, should decline with increasing queue size. Holling [1,2] categorized functional responses into three main types: 1) A linear functional response, when handling time is negligible, and the proportion of prey captured of the total number offered remains constant and independent of prey density (Type I). 2) A rectangular hyperbola when the consumption of prey is limited by satiation of predators, handling time and time spent hunting prey (Type II). 3) A sigmoid response (Type III) when learning behaviour occurs in the predator population with a consequent increase in the attack rate as more encounters with prey occur. During the present study, the prey-predator relationship exhibited a type II functional response that described the average feeding rate of a predator when it spent some time searching for the prey, and some time, exclusive of searching, and processing each captured prey item (handling time). The present study revealed that *Diplonychus indicus* consumed a good number of *Aedes* larvae which was corroborated with the findings of Ghosh and Chandra [21] who reported that *Laccotrephes griseus* consumed a good number of *Culex quinquefasciatus* larvae when the larval density ranged below 70 larvae per 250 mL of water, as increase in the larval density over this value caused a cessation in the feeding rate resulting in Type II functional response.

Other factors that may affect functional response are predator's search mode [30], development stage of

predator and prey [31], availability of prey refugees [32], and prey quality [33]. Predators' hunger/satiation has also been reported to be an important component of foraging behaviour that could have a significant effect on the feeding rate by influencing the motivation to search [34,35], and consequently functional responses [31,35-39]. Certain key factors determine the rate at which predation takes place through analysis of predator-prey models, which attempt to quantify the numerical and functional responses of predators to prey density [1,2,34,40]. Functional responses of predators to prey, especially strike success and foraging success, are complex and can be understood only by looking at each component separately. Further, Marin et al. [41] reported on certain factors that influence the predatory efficiency of predators should incontrovertibly be taken into consideration too, in addition to environmental factors.

5. CONCLUSION

The present investigation gives an essential idea with respect to the functional response of a predator, which play a vital role in the variation of predation efficiency on prey. This is a successful parameter in determination of actual prey predator mechanism, as the predatory efficiency of *Diplonychus indicus* was dependent on number of prey killed, attack rate, and handling time.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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