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Impact of Alternate Prey on the Prey Preference, Prey Switch Strategy and Predatory Performance of the water stick insect *Ranatra filiformis* Fabricius 1790 (Hemiptera: Nepidae)

Subramanian Arivoli[1], Samuel Tennyson[2*], Miriam Cecilia Vassou[3], Grace Marin[4], Raja Kalaivani[1] and Elangovan Vigneshkumar[1]
[1]Department of Zoology, Thiruvalluvar University, Vellore (Tamil Nadu), India.
[2]Department of Zoology, Madras Christian College, Chennai (Tamil Nadu), India.
[3]Department of Zoology, Periyar E.V.R. College, Tiruchirappalli (Tamil Nadu), India.
[4]Department of Zoology, Scott Christian College, Nagercoil (Tamil Nadu), India.

(Corresponding author: Samuel Tennyson)*

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ABSTRACT: The present work centers on the hypothesis of whether a predator can switch from one prey to another in a mixed prey population. The present endeavor is to clarify the same using nymphal stages of the water stick insect *Ranatra filiformis* with the prey species comprising of *Culex* and *Chironomous* larvae as a mixed prey population. To investigate the test hypothesis of this study, as well as density-dependent predation of *Ranatra filiformis* nymphs, this experiment was designed. *Ranatra filiformis* nymphal stages first and second, and from third to fifth were categorized as 'small and large predators', respectively. The nymphal predators were divided into four densities, *viz*., 4, 8, 12 and 16. The effectiveness of predation was investigated at prey densities of 50, 100, 150, and 200 of both *Culex* and *Chironomous* larvae. The first and second instar of the prey were categorized as 'small prey', while their third and fourth instar as 'large prey'. Four experimental design (ED) were set up to study predator-prey interaction, *viz*., ED1 (small predator and small prey), ED2 (small predator and large prey), ED3 (large predator and small prey), and ED4 (large predator and large prey). Each predator density of each experimental design were introduced into a single container with one litre of dechlorinated tap water, and was offered the above mentioned prey densities with an equal ratio of mixed prey population of *Culex* and *Chironomous*. The number of prey fed in ED1, ED2, ED3 and ED4 were noted after one hour and thereafter 24 hours. Overall, in ED1, *Chironomous* was preferred after one hour, and both *Culex* and *Chironomous* after 24 hours, and in general, small predators preferred *Chironomous*; in ED2, *Chironomous* was preferred after one hour, and *Culex* after 24 hours, and in general, small predators preferred *Culex* and *Chironomous*; in ED3, *Chironomous* was preferred after one hour, and *Culex* after 24 hours, and in general, large predators preferred both *Culex* and *Chironomous*; and in ED4, in general, large predators preferred *Chironomous*. When prey density was increased, predation by small predators was more pronounced in 24 hours, and by larger predators on small prey it fluctuated slightly after one hour, but increased predation was observed after 24 hours; and in the case of larger prey, predation fluctuated after one hour, but after 24 hours, a steady increased predation was observed. Secondly, the small and large predators preferred *Chironomous* after one hour, and both *Culex* and *Chironomous* after 24 hours. Thirdly, maximum predation by the small predators on small prey was at 150 and 200 prey density after one and 24 hours, respectively. In the case of small predators on large prey, and for large predators on small prey it was at 200 prey density for both one and 24 hours. Whereas, for large predators on large prey, it was at 100 and 200 prey density after one and 24 hours, respectively. The current investigation explained about the scenario of the predatory performance, as well as prey preference and prey switch by *Ranatra filiformis* nymphs in a mixed prey population.

Keywords: *Ranatra filiformis*, *Culex*, *Chironomous*, predator-prey relationship, predatory performance, predator density, prey density, prey preference, prey switch.

INTRODUCTION

Aquatic bugs of the family Nepidae prefer lentic habitats and possess long and slender stick-like legs. Their prey consumption pattern and predatory tactics involve sit-and-wait strategy, in comparison to the belostomatid bugs, which actively hunt for prey (Saha *et al*., 2007a,b).

The water stick insect genus *Ranatra* has a worldwide distribution, with about a half of its species richness in Southeast Asia (Polhemus and Polhemus 2012, 2013). Members of the genus *Ranatra* are found in tangled aquatic plant growth, and feed on a variety of aquatic prey, hitherto mosquito larvae are among the most frequently preferred ones (Menke, 1979). *Ranatra* species are ambush hunters that sits quietly on the aquatic plant and waits until its prey passes by, and then grabs the prey, injects a digestive fluid and sucks out the body contents. *Ranatra filiformis* is found in tangled plant growth or debris, where they are difficult to be detected due to their stick like appearance, hence called 'water stick insect'. It exhibits 'sit and wait in ambush' method of predation. With the help of its raptorial forelegs, they hold the prey firmly and inserts its rostrum into it and sucks the contents. *Ranatra filiformis* is an exterodigester and its prey capture sequence comprises of excitement, direction of the raptorial forelegs, capture of the prey, solidification of the grasp on the prey, exploration of the prey with the rostrum, insertion of the stylets, sucking, and discarding the prey (Marin *et al*., 2021). *Ranatra filiformis* holds the prey in the rostrum firmly and when any other prey reaches its attacking distance, it makes the attack on the latter too using its raptorial forelegs, thereby leaving the first prey free (Muthukrishnan, 1986).

The basics in predator prey relationship are to evaluate the functional response of a predator which reflects on the function relating to the number of prey consumed per unit time by a single average predator (Oaten and Murdoch 1975). Studies concerning aquatic insect's predator prey relationship often involves mosquito larvae as prey (Nasrabadi *et al*., 2022). In a predator-prey system, prey consumption by the predator can be considered as a direct effect. Presence of the additional species alters the effects of direct interactions between mosquito and insect predators, eventually affecting the regulation of mosquito population (Kundu *et al*., 2014). The preference for alternative prey displayed by predatory insects raises a concern for the effective regulation of the mosquito population, since it lowers the possibility of selection of mosquito prey (Blaustein and Chase 2007; Juliano, 2009). Apparent competition involves additional prey species, commonly termed the alternative prey, which shares the same predator with the key prey (mosquito) (Holt and Polis 1997). Consequently, the predator-prey system involving more than two species becomes complex in terms of food web properties. An increase in species number results in a corresponding increase in the number of links and connectance value, and thus increases the complexity of the system. If mosquito consumption by an insect predator is considered as a direct effect, an alternative prey would induce indirect effects, thereby altering the regulation of mosquito effectively (Kundu *et al*., 2014). *Ranatra filiformis* is an efficient predator on *Culex* larvae (Venkatesan *et al*., 1995), and studies on its predator prey relationship have been reported (Arivoli and Venkatesan 1995; Amsath, 2003; Mary and Venkatesan 2006; Marin *et al*., 2021). The present work centers on the hypothesis of whether a predator can switch from one prey to another in a mixed prey population. The present endeavor is to clarify the same using nymphal stages of the water stick insect *Ranatra filiformis* with the prey species comprising of *Culex* and *Chironomous* larvae as a mixed prey population.

MATERIALS AND METHODS

Ranatra filiformis. Adults of *Ranatra filiformis* collected from freshwater bodies of Vallimalai area, Vellore, Tamil Nadu, India using an insect net with a 200-m mesh size were transported to the laboratory and reared in glass aquariums (30" × 20" × 20") filled with pond water (10L). To recreate natural settings, gravels and few twigs of *Hydrilla* species were placed within the aquarium, which acts as substratum to the water stick insects. The insects were maintained at room temperature (30±2°C) with a photoperiod of 12 hours light: 12 hours dark cycle, and were fed with *Culex* larvae on a regular basis. After copulation, the eggs laid on the twigs of *Hydrilla* were isolated from the aquarium and transferred to small troughs for the emergence of nymphs.

***Culex* and *Chironomous* larvae.** *Culex* immatures collected from sewage with the aid of a ladle were transported in plastic containers to laboratory, and thereafter moved to enamel larval trays till adult emergence. The adults of *Culex* were fed on a 10% glucose solution in water, and their cyclical generations were kept separately in two-foot mosquito cages with a mean room temperature of 27±2°C and a relative humidity of 70-80% inside an insectary. The female mosquitoes were provided a blood meal, and they laid eggs in ovitraps placed within the cages for the mosquitoes, which were then moved to the larval rearing chamber, and kept in enamel larval trays. Larval food (dog biscuits and yeast 3:1) was provided to larvae. With regard to *Chironomous* larvae, they were also collected from the sewage, and transported to the laboratory in polythene cover. In the laboratory, they were reared using crude wet cow dung extract as nutrient sources. The water level was maintained throughout the rearing periods with a temperature of 27±2°C. Eggs laid by gravid adults were transferred to a separate tray for larvae to hatch, and thereafter reared up to the adult stages.

Experimental design. To investigate the test hypothesis of this study, as well as density-dependent predation of *Ranatra filiformis* nymphs, this experiment was designed. *Ranatra filiformis* nymphal stages first and second, and from third to fifth were categorized as 'small and large predators', respectively. The nymphal predators were divided into four densities, *viz.,* 4, 8, 12 and 16. The effectiveness of predation was investigated at prey densities of 50, 100, 150, and 200 of both *Culex* and *Chironomous* larvae. The first and second instars of the prey were categorized as 'small prey', while their third and fourth instars as 'large prey'. Four experimental design (ED) were set up to study predator-prey interaction, viz., ED1 (small predator and small prey), ED2 (small predator and large prey), ED3 (large predator and small prey), and ED4 (large predator and large prey). The small predator with density of 4 of ED1 were introduced into a single container with one litre of dechlorinated tap water, and was offered with an equal ratio of mixed prey population of 50 density. The number of prey fed by the small predators were noted after one hour and thereafter 24 hours. Control had the same number of replicates but lacked predators to ensure mortality does not occur in any prey. A total of five trials were performed to determine the optimum prey density that the nymphal predators preferred. The same protocol was followed for each experimental design, with each predator density as well as for each prey density. Data on prey death rate by the nymphs of *Ranatra filiformis* were analysed with ANOVA, and statistical differences were determined to be significant at P<0.05 level (SPSS, 2021).

RESULTS AND DISCUSSION

No prey mortality was reported in any of the control sets.

ED1: Small predator and small prey. At predator density of 4, increased and decreased predation was observed at prey density of 100 (7.5±2.0), and 50 (1.0±1.2) after one hour, and at a prey density of 150 (52.2±13.6), and 50 (3.2±1.1) after 24 hours, respectively. At predator density of 8, increased and decreased predation was noted at prey density of 150 (9.0±5.5), and 50 (1.0±1.2) after one hour, and again at the same prey density (45.2±22.6) and (6.6±7.5) after 24 hours, respectively. At predator density of 12, increased and decreased predation was observed at prey density of 200 (9.8±2.3), and 50 (1.4±1.3), after one hour; and again at the same prey density (86.4±26.1) and (3.2±0.7) after 24 hours, respectively. At predator density of 16, increased and decreased predation was noted at a prey density of 150 (17.4±11.7) and 50 (4.2±3.1) after one hour; and at a prey density of 200 (79.8±28.3), and 50 (5.0±2.8) after 24 hours, respectively (Table 1). The prey preferred after one hour at prey density of 50 and 100 was *Chironomous.* At prey density of 150, equal number of *Culex* and *Chironomous* was preferred, and at 200 prey density, *Culex* was preferred. After 24 hours, *Chironomous* was preferred at prey density of 50 and 100, and *Culex* at 150 and 200. Overall, it was noted that *Chironomous* was preferred after one hour, and both *Culex* and *Chironomous* after 24 hours (Fig. 1). In general from ED1, small predators preferred *Chironomous.*

ED2: Small predator and large prey. At predator density of 4, increased and decreased predation was observed at prey density of 100 (9.4±4.0), and 50 (1.8±2.6) after one hour, and at a prey density of 200 (47.6±14.9), and 50 (1.4±1.3) after 24 hours, respectively. At predator density of 8, increased and decreased predation was noted at prey density of 150 (9.0±7.5) and 50 (1.0±1.2) after one hour, and at a prey density of 200 (53.2±18.8) and 50 (1.8±1.1) after 24 hours, respectively. At predator density of 12, increased and decreased predation was observed at prey density of 200 (12.4±2.9) and 50 (2.4±2.5), after one hour; and again at the same prey density (92.8±51.6) and (2.0±1.8) after 24 hours, respectively. At predator density of 16, increased and decreased predation was noted at a prey density of 200 (20.4±9.0) and 50 (1.6±1.3) after one hour; and again at the same prey density (33.6±14.4) and (3.6±2.4) after 24 hours, respectively (Table 2). The prey preferred after one hour at prey density of 50 was *Chironomous*, and at 100 it was *Culex*. At prey density of 150 and 200, a slight increase in preference towards *Chironomous* was observed. After 24 hours, *Chironomous* was preferred at prey density of 50 and 100, and at 150 and 200, a slight increase in preference towards *Culex* was noted. Overall, it was noted that *Chironomous* was preferred after one hour, and *Culex* after 24 hours (Fig. 2). In general from ED2, small predators preferred *Culex* and *Chironomous*.

ED3: Large predator and small prey. At predator density of 4, increased and decreased predation was observed at prey density of 150 (12.6±6.3) and 200 (0.6±4.0) after one hour, and at a prey density of 200 (59.2±36.6), and 50 (9.0±4.1) after 24 hours, respectively. At predator density of 8, increased and decreased predation was noted at prey density of 150 (9.0±5.5), and 50 (1.0±1.2) after one hour, and at a prey density of 200 (45.2±22.6) and 50, 100 (11.8±3.9, 11.8±2.7) after 24 hours, respectively. At predator density of 12, increased and decreased predation was observed at prey density of 200 (27.6±8.5), and 100 (5.0±3.8), after one hour; and at prey density of 200 (71.8±16.9) and 50 (12.0±7.4) after 24 hours, respectively. At predator density of 16, increased and decreased predation was noted at a prey density of 50, 200 (9.0±5.7, 9.0±3.6) and 100 (5.6±3.4) after one hour; and at prey density of 200 (42.2±10.7) and 50 (12.8±5.1) after 24 hours, respectively (Table 3). After one hour, both *Culex* and *Chironomous* were preferred at prey density of 50 and 100, and at prey density of 150, a slight increase in preference towards *Chironomous* was observed, and at 200 prey density, *Chironomous* was preferred. After 24 hours, both *Culex* and *Chironomous* were preferred at prey density of 50 and 100, and *Culex* at 150 and 200. Overall, it was noted that *Chironomous* was preferred after one hour, and *Culex* after 24 hours (Fig. 3). In general from ED3, large predators preferred both *Culex* and *Chironomous*.

ED4: Large predator and large prey. At predator density of 4, increased and decreased predation was observed at prey density of 100 (13.6±8.4) and 50 (2.0±0.8) after one hour, and at a prey density of 200 (44.8±15.4), and 50 (9.2±9.4) after 24 hours, respectively. At predator density of 8, increased and decreased predation was noted at prey density of 100 (19.8±9.2) and 50 (2.0±2.2) after one hour, and at a prey density of 200 (78.6±36.6) and 50 (9.0±6.6) after 24 hours, respectively. At predator density of 12, increased and decreased predation was observed at prey density of 150 (22.0±10.1), and 100 (5.2±1.7), after one hour; and at prey density of 200 (89.0±29.9) and 50 (14.0±3.4) after 24 hours, respectively. At predator density of 16, increased and decreased predation was noted at a prey density of 50 (17.0±4.0) and 100 (6.0±2.0) after one hour; and at prey density of 150 (72.2±2.5) and 150 (16.6±20.5) after 24 hours, respectively (Table 4). The prey preferred after one hour at prey density of 50 and 150 was *Chironomous*, and at 100 and 200, a slight increase in preference towards *Chironomous* was observed. After 24 hours, a slight increase in preference towards *Chironomous* was observed at prey density of 50 and 100, and *Chironomous* at 150 and 200 (Fig. 4). Overall, as well as in general from ED4, large predators preferred *Chironomous*.

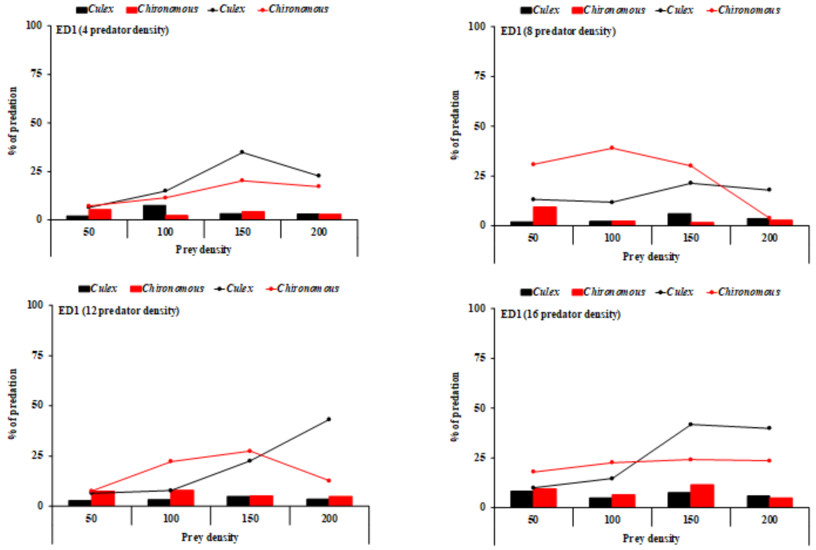

Fig. 1. Percentage of predation by small predator on small prey (Bar data and line data denotes 1 hour and 24 hours of exposure respectively).

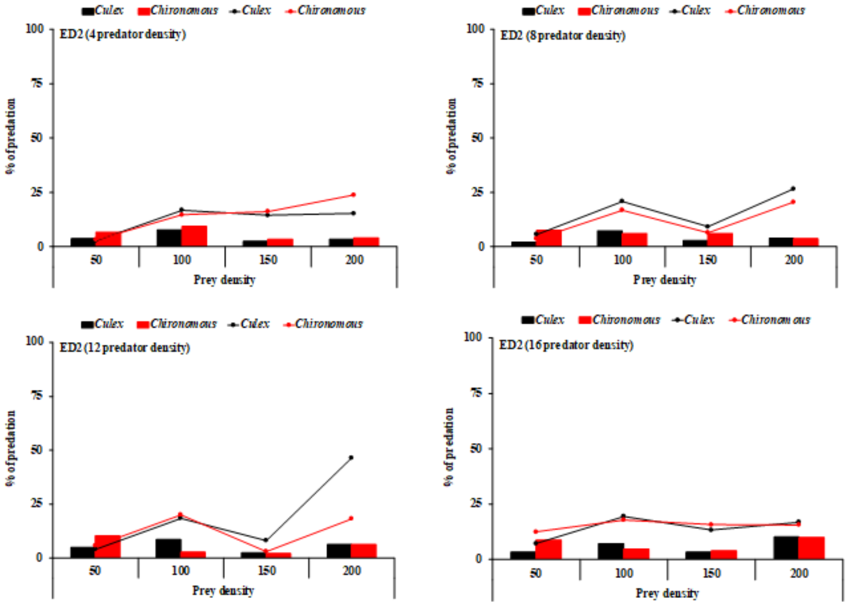

Fig. 2. Percentage of predation by small predator on large prey (Bar data and line data denotes 1 hour and 24 hours of exposure respectively).

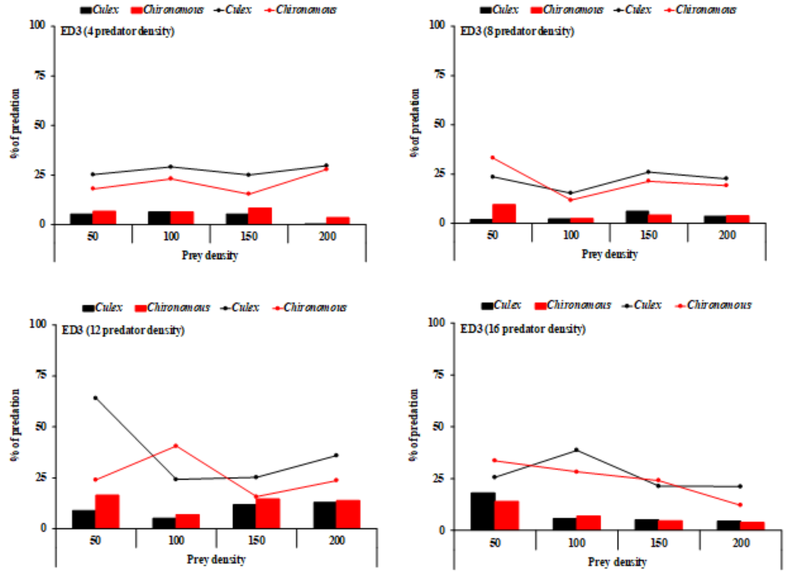

Fig. 3. Percentage of predation by large predator on small prey (Bar data and line data denotes 1 hour and 24 hours of exposure respectively).

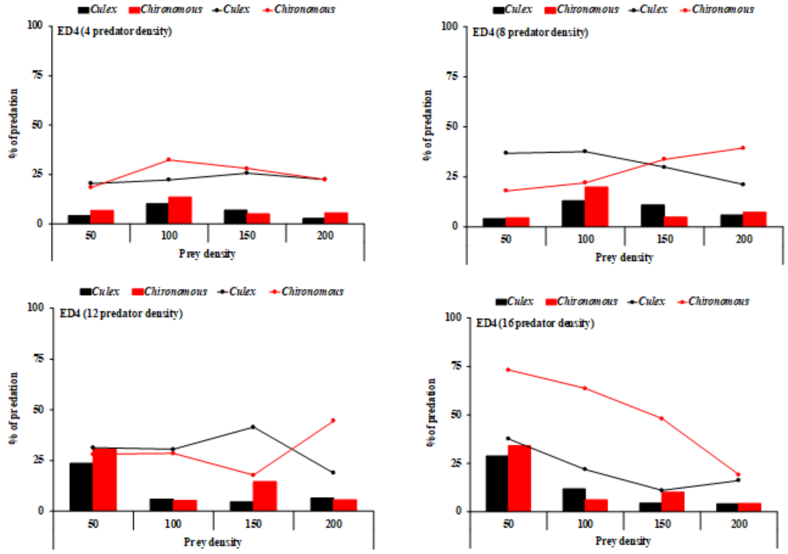

Fig. 4. Percentage of predation by large predator on large prey (Bar data and line data denotes 1 hour and 24 hours of exposure respectively).

Table 1: Predatory efficiency of small predators on small prey (ED1).

Predator density	**Prey density**							
	50		**100**		**150**		**200**	
	Culex	***Chironomous***	***Culex***	***Chironomous***	***Culex***	***Chironomous***	***Culex***	***Chironomous***
1 hour								
4	1.0±1.2	2.6±1.8	7.5±2.0	2.2±2.0	5.0±2.2	6.4±3.6	6.2±4.3	5.8±2.3
8	1.0±1.2	4.8±2.7	2.2±2.2	2.4±1.4	9.0±5.5	2.4±1.4	7.2±3.1	5.8±5.0
12	1.4±1.3	3.8±2.4	3.2±2.0	8.0±5.7	7.2±4.6	7.6±3.6	7.0±3.5	9.8±2.3
16	4.2±3.1	4.8±3.3	4.8±2.3	6.4±3.8	11.4±5.7	17.4±11.7	11.6±5.4	9.8±3.7
24 hours								
4	3.2±1.1	3.6±2.6	15.0±2.8	11.4±5.3	52.2±13.6	30.4±10.7	45.2±14.6	34.4±18.1
8	6.6±7.5	15.4±6.2	11.8±2.7	39.0±15.8	32.2±13.5	45.2±22.6	36.0±9.9	7.6±3.8
12	3.2±0.7	3.8±1.3	7.8±3.0	22.2±8.1	33.8±14.2	41.4±7.8	86.4±26.1	25.2±12.4
16	5.0±2.8	9.0±8.0	14.6±6.6	22.6±8.3	62.8±32.2	36.4±21.2	79.8±28.3	47.2±34.4
F value	7.529		3.839		8.966		11.203	
P value	0.008*		0.057*		0.005*		0.002*	

*Values significant at P<0.05

Table 2: Predatory efficiency of small predators on large prey (ED2).

Predator density	Prey density							
	50		100		150		200	
	Culex	*Chironomous*	*Culex*	*Chironomous*	*Culex*	*Chironomous*	*Culex*	*Chironomous*
1 hour								
4	1.8±2.6	3.4±2.3	7.8±5.0	9.4±4.0	3.8±3.7	5.2±3.2	7.0±4.3	8.0±5.1
8	1.0±1.2	3.8±2.7	7.4±4.9	6.0±2.0	4.2±2.4	9.0±7.5	7.8±5.2	7.2±4.1
12	2.4±2.5	5.2±2.2	8.6±5.1	2.6±1.4	3.6±3.3	3.2±2.5	4.7±2.5	12.4±2.5
16	1.6±1.3	4.4±1.0	7.2±2.7	4.6±1.8	5.0±2.8	5.8±1.7	20.4±9.0	20.0±8.6
24 hours								
4	1.4±1.3	1.6±2.5	16.8±11.6	14.6±7.5	21.6±8.0	24.4±15.1	30.6±13.5	47.6±14.9
8	2.8±2.1	1.8±1.1	20.8±13.9	16.8±5.4	13.8±2.1	9.6±3.3	53.2±18.8	41.0±9.6
12	2.0±1.8	2.8±0.9	18.4±14.9	20.0±7.0	12.2±7.1	4.6±2.1	92.8±51.6	36.4±9.4
16	3.6±2.4	6.2±9.4	19.4±11.9	17.8±9.4	19.8±14.4	23.6±10.6	33.6±14.4	31.2±13.8
F value	6.470		14.521		0.780		14.446	
P value	0.014*		0.001*		0.382		0.001*	

*Values significant at P<0.05

Table 3: Predatory efficiency of large predators on small prey (ED3).

Predator density	Prey density							
	50		100		150		200	
	Culex	*Chironomous*	*Culex*	*Chironomous*	*Culex*	*Chironomous*	*Culex*	*Chironomous*
1 hour								
4	2.6±0.4	3.4±1.8	6.2±0.7	6.2±2.4	7.8±5.2	12.6±6.3	0.6±4.0	7.0±2.4
8	1.0±1.2	4.8±2.7	2.2±2.2	2.4±1.4	9.0±5.5	6.4±3.3	7.2±5.5	7.6±3.3
12	9.0±2.1	8.2±1.4	5.0±3.8	7.0±5.5	11.8±2.7	21.8±10.2	13.0±4.2	27.6±8.5
16	9.0±5.7	7.0±1.7	5.6±3.4	7.0±1.7	7.6±2.4	7.0±2.8	9.0±3.6	7.8±9.3
24 hours								
4	12.6±1.8	9.0±4.1	29.0±11.5	23.0±11.8	37.6±12.9	23.2±15.4	59.2±36.6	55.6±12.5
8	11.8±3.9	16.6±5.0	15.4±6.2	11.8±2.7	39.0±15.8	32.2±13.5	45.2±22.6	38.4±8.6
12	32.0±24.8	12.0±7.4	24.2±6.7	40.6±10.0	37.8±13.9	23.6±22.4	71.8±16.9	47.4±37.3
16	12.8±5.1	16.8±7.6	38.6±23.1	28.2±16.7	32.0±20.0	36.2±15.0	42.2±10.7	24.2±6.8
F value	0.983		17.130		8.638		13.440	
P value	0.326		0.001*		0.005*		0.001*	

*Values significant at P<0.05

Table 4: Predatory efficiency of large predators on large prey (ED4).

Predator density	Prey density							
	50		100		150		200	
	Culex	*Chironomous*	*Culex*	*Chironomous*	*Culex*	*Chironomous*	*Culex*	*Chironomous*
1 hour								
4	2.0±0.8	3.4±2.9	10.2±0.9	13.6±8.4	10.4±3.0	7.6±4.2	5.2±3.4	11.0±5.7
8	2.0±2.2	2.2±1.3	13.0±2.5	19.8±9.2	16.4±13.0	7.4±2.8	11.8±4.4	14.8±5.4
12	11.8±7.0	15.2±13.1	5.8±2.9	5.2±13.1	6.8±6.0	22.0±10.1	13.2±8.5	10.8±2.1
16	14.4±2.8	17.0±4.0	11.8±9.1	6.0±2.0	6.8±3.8	15.2±7.0	8.2±4.3	8.4±5.4
24 hours								
4	10.2±0.9	9.2±9.4	22.2±14.1	32.4±17.5	38.6±13.4	42.0±20.2	44.8±15.4	44.6±13.7
8	18.4±4.4	9.0±6.6	37.6±7.2	22.0±7.8	44.8±8.8	50.8±16.7	42.2±29.7	78.6±36.6
12	15.6±2.4	14.0±3.4	30.4±9.6	28.4±16.8	62.2±20.3	26.6±13.6	37.6±13.2	89.0±29.9
16	18.8±5.7	36.6±7.2	21.8±12.6	63.6±3.7	16.6±20.5	72.2±2.5	32.4±31.2	38.2±17.0
F value	14.150		0.038		0.061		4.462	
P value	0.001*		0.847		0.806		0.041*	

*Values significant at P<0.05

Prey selectivity by *Ranatra filiformis*, and the role of the alternative prey remained the test hypothesis in the present study. Alternative prey influences the outcome of predator prey interactions in aquatic communities. The presence of more than one prey creates an opportunity for the predator to exhibit selectivity, resulting in differential prey resource exploitation (Saha *et al*., 2020). Insect predators belonging to the family Nepidae commonly occurring in aquatic ecosystem together with mosquito larvae (Das *et al*., 2006; Kundu *et al*., 2014), exhibit mosquito prey selectivity against a single alternative prey, although it is more obvious that natural habitats will host multiple species as alternative prey (Saha *et al*., 2020). Coexistence of multiple prey increases obstruction in target prey regulation (Morris *et al*., 2001). Consumption of alternative prey reduces the vulnerability of mosquito prey to the insect predators, affecting the regulation of mosquito larvae (Saha *et al*., 2020). Chironomid larvae as an alternative prey reduce mosquito prey consumption by insect predators like water bugs (Aditya *et al*., 2004; Saha *et al*., 2009, 2010; Sivagnaname, 2009). The predation ecology of the insects may vary in a context dependent manner (Juliano, 2009), depending on the presence of the alternative prey (Saha *et al*., 2009, 2010), the habitat conditions (Saha *et al*., 2008, 2009; Dalal *et al*., 2019) and the search area (Dalal *et al*., 2020). A precise assessment of the functional role of the insects as predators of mosquitoes can be deduced through the study on prey consumption in the presence of alternative prey with the possibilities of several indirect interactions (Blaustein and Chase 2007), like apparent competition or intraguild predation (Kundu *et al*., 2014; Brahma *et al*., 2015a,b).

Predatory performance increases with increase in period of exposure, and the same was observed in the present study. Muthukrishnan (1986) reported prey death rate caused by *Ranatra* species, fluctuated at an interval of one hour and 24 hours with different densities of prey. In *Ranatra linearis*, prey density influenced the duration of the predation. In the present study, predation by small predators on small prey increased with increase in high prey density after one hour, and 24 hours of exposure; and when predated on large prey, the same trend followed except for minor fluctuations when the prey density increased. Hence, predation by small predators was more pronounced in 24 hours than in one hour. Predation by larger predators on small prey fluctuated slightly as the prey density increased after one hour, but increased predation was observed with increase in prey density after 24 hours, as they consumed more prey; and in the case of larger prey, increase in predation fluctuated after one hour, when prey density increased, but after 24 hours, a steady increased predation was observed when prey density was increased. Secondly, aquatic insect predators resort to multiple prey capture (Bailey, 1985). Blois and Cloarec (1983) reported that *Ranatra linearis* increased their selectivity in the presence of high prey population. The prey preference varied among the small and large predators in the present study. *Ranatra filiformis* nymphs, irrespective of their stage preferred large size *Culex* and *Chironomous* larvae. In the present study, the small and large predators preferred *Chironomous* after one hour, and both *Culex* and *Chironomous* after 24 hours. Thirdly, factors that influence prey selection are the absolute and relative densities of available prey types. According to density dependent selection, a predator should become selective when densities are high. This has been tested with functional response trials by Bailey (1985) on *Ranatra* species. Venkatesan *et al*. (1995) reported that adult *Ranatra filiformis* is an efficient predator of large sized *Culex* larvae and increase in prey density increases the predatory performance. Amsath (2003) reported that the rate of predation in *Ranatra filiformis* was more at higher prey densities (75 and 100) than at lower prey densities (25 and 50), and that the predator preferred second and third instar larvae of *Culex fatigans* than the fourth instar. An individual predator attacks more prey as prey density increases, as there exists a relationship between prey density and prey depletion which make the predators to kill and discard the prey significantly at high densities. In the present study, maximum predation by the small predators on small prey was at 150 and 200 prey density after one and 24 hours, respectively. In the case of small predators on large prey, and for large predators on small prey it was 200 prey density for both one and 24 hours. Whereas, for large predators on large prey, it was at 100 and 200 prey density after one and 24 hours, respectively. Ellis and Borden (1970) corroborated the same on the back swimmer *Notonecta undulata* that maximum number of prey killed per hour in relation to increased prey density.

Predation in aquatic insects are governed by a number of limiting factors, viz., predator's stage and performance, prey density, prey recognition, prey choice, prey size, prey switch, prey capture tactics, and multiple prey capture. Taking all the above stated criteria into consideration, the results of the present investigation reveals as to how the nymphal stages of *Ranatra filiformis* predated on the mixed prey species.

Predator size and performance. The response of a predator is strongly affected by its size and stage. Size related changes in attack rate and handling time introduce complex dynamics during predator-prey interactions. Maximum attack rate is found for small predators attacking small prey and larger predator attacking small prey and larger predator attacking large prey. This was noted in the present study. Secondly, the searching and feeding behaviour of aquatic predators generally change as prey 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 (1961) 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. The description of a predator's instantaneous, feeding rate or predatory impact, as a function of prey density, is its 'functional response'. 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 (Murdoch and Oaten 1975). Functional responses are evaluated by 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.

Prey density. The present study gives an essential idea with respect to the variation in the predatory performance with change in the prey density. This is an effective parameter in determination of actual feeding rate in field condition as it is conceivable to decide the quantity of predators (predator density) that ought to be introduced in accordance to prey density and available volume of search area in the field (Marin *et al*., 2021). In the present study, maximum predation was noted at the highest prey density of 200 after 24 hours of exposure period. Predators react to changes in density of prey. Venkatesan *et al*. (1995) reported that predation in *Ranatra filiformis* is highly influenced by prey density to which the predator gets exposed. The response of predators to changes in prey density varies. When the predator attacks more, prey density decreases. At higher prey densities, the predator spends 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 prey density. Holling (1959a,b) stated this as a very effective parameter in the determination of actual feeding rate in the field condition as it is possible to determine the number of predators that should be introduced in response to a particular prey density and available volume of search area.

Prey recognition. The sit-and-wait strategy is characteristic of *Ranatra* (Blois and Cloarec 1983; Bailey, 1986) that require that the prey be pursued once it arrives in the vicinity of the predator. *Ranatra* species can perceive and locate a potential prey either by visual or by mechanical stimulation alone, but, during certain times, the relative importance of mechanoreception decreases while the relative importance of perception of visual stimuli increases (Cloarec, 1976). Hence, the estimation of predator-prey distance is necessary before a strike is to be elicited, to ensure enough successful captures. Cloarec (1986) reported that when two different sized targets were presented at the same distance, both monocular and control subjects prefer the larger target, even though monocular animals choose the larger target consistently. This was observed in the present study. Based on the wriggling movement of *Culex* and *Chironomous* larvae, the predator recognized the prey easily and fed on them.

Prey choice. Tested hypotheses concerning relationship between predator foraging mode and pattern of prey selection was reported by Cooper *et al*. (1985) on several freshwater predators. The presence, and movement and distribution of *Chironomous* larvae, may have influenced the prey selection by *Ranatra filiformis* which is a crucial factor in the outcome of the alternate prey species interaction as reported by Saha *et al*. (2020). The coefficient of apparent competitor, *Chironmous* larvae buffered the vulnerability of mosquito larvae. Saha *et al*. (2020) reported that the presence of alternative prey influenced the mosquito prey selectivity of the predatory insects. Selectivity of alternative prey by *Ranatra filiformis* and *Ranatra elongata* varied with prey species identity. Further, prey preference is also coupled with the phenomenon of prey switching in accordance with the availability and non-availability as well as the proportion of the preferred prey species. In the present study, the small and large predators preferred *Chironomous* after one hour, and both *Culex* and *Chironomous* after 24 hours.

Prey size. When a predator has a choice of prey differing only by size, it often selects the biggest ones, however, chances are where the smaller items are captured too. Certain factors, therefore, seem to channelize selection of a given size class. One of the major factors that can influence prey selection, according to optimal foraging theory, is the profitability of the prey. This is the function of the energy gained from the prey relative to the energy and the time spent foraging. When the most profitable prey type is abundant and easily found, the predator should specialize on that prey type, under the influence of prey size. Holling's concept refers to the optimum prey size that a predator can handle with its raptorial foreleg (Holling, 1961). Difference in size between two groups of prey is marked more important, as the predator's preference is for the larger prey. *Ranatra* species at all stages for development choose a particular size of prey. *Ranatra linearis* predate large prey than small ones, and more towards the size at low prey densities than at high prey densities (Blois and Cloarec 1983). The prey death rate is higher with large sized prey than with small sized. The behaviour of the predator could be concentrated on the selection of the prey and eating ability operates secondarily depending upon the efficiency and the nature of the prey (Venkatesan and Sivaraman 1984). In the present study, predation on small prey by small predators was pronounced, and by larger predators on small and large prey, it fluctuated slightly, but a steady increased predation was observed thereafter.

Prey capture tactics. *Ranatra* species exhibit sit and wait predaceous habit mainly to conserve energy and predator prey distance. The most optimal foraging models have examined prey selection using predators considerably larger than their prey, and the role of receptors in *Ranatra linearis* where during their development they can perceive a potential prey either by visual or by mechanical stimulation alone, although in normal circumstances information from both series of receptors combine before eliciting strike (Blois and Cloarec 1983). It is also in accordance to the work of Cloarec (1973) who has put forward the predatory performance of *Ranatra linearis* illustrating its hit distance, foreleg movement and its strike efficiency in the light of deprivation of prey and the role of mechano and chemo receptors. The same was observed in the present study.

Multiple prey capture. Some predators resort to multiple prey capture and are able to handle prey in such a way that additional prey can be caught without having to abandon the previous one. Muthukrishnan (1986) stated that when the prey was held in the rostrum firmly, or when any other prey reached its attacking distance, *Ranatra* made the attack on the latter using its raptorial forelegs, thereby leaving the first prey free. The same was observed in the present study.

Prey switch. Predators maintained a pattern of predatory performance with prey items in prey combinations. A standard way of describing the intensity of the predation by a single individual of a predator species on a single prey species is the functional response, the function relating to the number of prey consumed per unit time by a single average predator to the size of the prey population. Clearly, this function may depend on other prey species that may appear to the predator. Such predators attack a variety of prey and the idea is that they tend to feed most heavily upon the most abundant species (Murdoch, 1969). As this particular prey species declines in number, partly owing to the predation, the predator switches the great proportion of its attack to another prey which has become the most abundant. If one assumes that the predator searches randomly at constant speed for randomly distributed prey, the predator should not show the switching mechanism. Contrastingly, predators showed a gradual change in the preference of a prey species to the other in the present study. Probably switching of the prey (i.e. number of prey fixed in each ratio) and also the duration of exposure may have an impact on the predatory performance of *Ranatra filiformis* in the present study. It is of interest to hypothesize whether prey switch has any effect on predatory performance of *Ranatra fililformis*, which remains unanswered, opening doors for further studies.

CONCLUSIONS

The current investigation clearly explained about the scenario of the predatory performance as well as prey preference and prey switch by the nymphal stages of *Ranatra filiformis* in a mixed prey population.

FUTURE SCOPE

Future experimental work comprising of different alternate prey, different prey and predator density, different water volume, with nymphal and adult stages of other predatory aquatic insects will play a pivotal role in throwing much spectrum of light on the predatory performance.

Conflict of Interest. None

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