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Bradinopyga geminata (Anisoptera: Libellulidae) as a predator of *Aedes aegypti* immatures (Diptera: Culicidae)

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Abstract
Predatory potential of 12th instar larvae of *Bradinopyga geminata* on *Aedes* mosquito immatures was observed, by exposing two different prey-predator combinations (Prey: Predator; 200:1 and 1000:5). One and five 12th instar larvae of *B. geminata* were provided with 200 (SET A) and 1000 (SET B) I, II, III & IV instars of *Aedes aegypti* larvae as prey, for a period of 24 hr in plastic containers containing 1 and 5 litres of water respectively. The number of *Ae. aegypti* larvae consumed by *B. geminata* larvae were noted through one day, at an interval of 3 hours. To maintain the prey density, same number of larvae was replenished. In the daily feeding rate experiment the consumption showed a peak during the 9thhour, irrespective of the instar stages. Predation rate of *B. geminata* was more for I instar, The predatory impact values for I instar in both Set A and B were 4.12+0.05 and 3.6+0.02 respectively, and were significant *(P<0.01)*. The comparative clearance rate for Set A and B was highly significant for the first instar *(P<0.01)*. This study revealed that *B. geminata* larvae is an efficient predator of mosquito larvae. The rate of consumption was dependent on the size of the prey and the density of the predator. The predatory impact of *B. geminata* was more for the first instar *Ae. aegypti*, owing to its size and energy requirements. To conclude, *B. geminata* is an efficient bio-control agent for container breeding *Ae. aegypti* and can be an effective tool in the integrated vector control programme.

Keywords: *Bradinopyga geminata, Aedes aegypti*, predator, biological control

1. Introduction
Dengue has been a major public health problem in almost all tropical and subtropical countries, and currently there is no effective vaccine. Mosquitoes are important insects not only as nuisance biters but also as vectors of important diseases such as malaria, filaria and dengue, particularly in the tropics. In view of this, renewed interest in biological control agents, particularly aquatic predaceous insects that inhibit mosquitoes' in their breeding sites could provide suitable solution, and could be included in integrated vector management (IVM) program. The control of mosquito in their larval stage is more efficient in the integrated mosquito management. During the immature stage, mosquitoes are relatively less mobile; remaining more concentrated than they are in the adult stage [1].

Biological control is generally defined as the use of natural enemies including pathogens, parasites and predators in reducing pest populations in natural habitats. The incorporation of biological measures in an integrated mosquito control program requires a careful selection of the antagonistic organism, so that the human protection is achieved without affecting the biodiversity and without inducing ecological problems. Experimental studies over the last century revealed a great diversity of living organisms, including microbes, fungi, protozoa, nematodes, invertebrate and vertebrate predators, as promising mosquito control agents. All groups of organisms that have been tested as potential bio-control agents, (including aspects on field trials, handling, transporting and laboratory activity testing of the isolates on different groups of vectors) have been extensively discussed [2].

The predatory insects like damselfly (Odonata: Anisoptera) and dragonfly (Odoanata: Zygoptera) larvae are important predators of many microinvertebrates including the larvae of mosquito [3-5]. Many experimental studies were reported with different species of damselfly and dragonfly larvae, to control mosquito larvae throughout the world [6-18]. and also predation on mosquito larval habitats in tree-holes like the larvae of damselfly [19, 20]. Periodic augmentative release of predaceous larvae of *Crocothemis servilia* was able to suppress mosquito larval population in Myanmar [9]. *Enallagma civile* [7], *Sympetrum striolatum* [21], *Orthemis ferruginea* [22],

Pantala hymeneae [5], *Bradinopyga geminata* and *Ceriagrion coromandelianum* [17, 18]. have been recorded as predators of mosquito larvae.

Usually mosquito populations are controlled by various agents, viz; commonly used synthetic chemical [23, 24], microbial [25, 26]. and fungal insecticides [27-30]. nematodes [31], aquatic beetles [32] and backswimmers [33-37]. with particularly adequate life history characteristics and metapopulation structure [38, 39]. The control system is able to incorporate any agents in mosquito population management, such as release of predators, new genetic methods or inexpensive repellents and oviposition deterrents [40].

The extensive use and the lack of adequate knowledge however, had a tremendously destructive impact on the environment and the wild life, including fish, birds, arthropod predators, insect pollinators and soil micro-organisms. The concept of using living organisms in controlling mosquitoes dates back to 19th century, when the first attempts were made to introduce dragonflies as predators in mosquito breeding places [41]. Mean-while, it was noticed that several other organisms, aquatic or terrestrial, could consume mosquitoes as food. Very few, however, were effective and have been considered as possible biological control agents [40]. Since predation is considered as the strongest selection pressure in natural ecosystems it is expected that many organisms such as mosquitoes in water ecosystems have evolved a variety of adaptive ways to avoid predation such as crypsis, chemical defences (for *Culex* predated by *Gambusia affinis* [42]; for tadpoles predated by odonates [43]) and behavioural strategies [44]. *Bradinopyga geminata* has recorded in metal drums, which were mainly brownish to blackish cylindrical iron barrels [45]. Predatory potential of *B. geminata* and *C. coromandelianum* on *Aedes aegypti* larvae consumption rate was maximum in the first hour observation for all instars and low intake was observed in subsequent hours [17].

In view of these facts, 12th instar of *B. geminata* larvae were evaluated for its role as an active feeder and able to consume mosquito larvae.

2. Material and methods

2.1. Collection of predator

The dragonfly larvae were collected from the cement pond ecosystem which are located in Tamil Nadu Agricultural College campus Madurai (latitude 9º58'0.8.25ºN, 78º2'11.66ºE), Madurai by using a standard larval dipper with long-handled nets with 15cm diameter x 30cm long muslin sleeves. The predators were transported alive from the field to the Centre for Research in Medical Entomology (CRME) laboratory in plastic boxes half-filled with water and debris from the breeding sites. In the laboratory, the predators were washed with clean water and sorted into small plastic trays, half-filled with de-ionized water and maintained in small aquaria filled with tap water. The specimens were identified using standard keys [46].

One and five 12th instar of *B. geminata* larvae were provided with 200 and 1000 I - IV instars of *Aedes aegypti* larvae as prey for a period of 24 hr in plastic container (6 litre capacity) containing 1 litre and 5 litres of water respectively. The water of the habitat of *B. geminata*, was used in the experiments after sieving through a net (>500 mesh) to exclude any larvae of other predator species. Abrupt changes in the quality of holding water during rearing and experimentation were avoided. The predation experiment was conducted on separate days for each instars and each with three replications. A control group was maintained for each experiment.

The number of *Ae. aegypti* larvae consumed by *B. geminata* larva was noted through one day at an interval of 3 hours for I-IV instars. At each 3 hour interval, the water of the experimental sets were poured through a fine mesh sieve to collect all mosquito larvae. After counting the number of consumed larvae every 3 hours, the same number of larvae were replenished in the container to maintain the prey density. To observe the daily feeding rate, the experiment was commenced at 6 a.m. of a day and was completed at 6 a.m. of the next day. To determine the predatory impact, the method adopted by Aditya *et al.* (2006) [47] and Nabaneeta *et al.* (2010) [48] was as follows:

$$PI = \frac{\sum_{n=1}^{7} PE}{T}$$

PI = Predatory impact (No. of prey larvae / hr)
PE = % of prey eaten or killed
T = Time in hrs

The clearance rate (CR) reflects the combined effect of search ability, killing and consumption by the predator and prey evasion, in unit time and space. CR was determined as stated by Gilbert & Burns (1999)[49].

$$CR = \frac{V\ (In\ P)}{TN}$$

CR= Clearance rate of predators (% of prey killed litres/day/predator); V = Volume of water; P = % of prey killed; T= Time (in day); N = No. of predators

1.1. Collection of prey

The immatures of the *Aedes aegypti* mosquitoes were collected from CRME Mosquito Colony. The laboratory colony was maintained at 25–30 ºC temperature with supplementary food consisting mixture of protein biscuit (60%) and dried Yeast powder (40%). Larvae of each instar were continuously available for the experiments. Thus, I to IV instar larvae of these mosquitoes were used in the experiments in the present study.

3. Data analysis

The significant differences of predator and prey level were tested using t-test. The descriptive statistics of Mean and SE were used in different replicates. The experimental data were analyzed using SPSS ver.16 software and MS Excel was used to normal data quality making and graphical presentations.

4. Results

The impact of prey density and predatory potential of 12th instar of *B. geminata* was recorded for a period of 24 hours at an interval of three hours. This was conducted in two sets. In the first Set A, each predatory was provided with 200 larvae and in the other Set B, 1000 larvae were provided to 5 predators. The average values were calculated for the three replicates. The results of consumption in both the sets were provided in Table 1 and Figures 1 &2. From the above tables and figures, it is apparent that the consumption showed a peak

during the 9th hour of the day, irrespective of the instar stages. The second peak was observed at 3 a.m. in both the cases. Predation rate of *B. geminata* was more when it was exposed to I instar, while it showed a declining trend for II to IV instars indicating its preference to I instar larvae. Apart from the major and minor peaks, smaller predatory potential was observed during other hours of the day. This was similar in both Sets of experiments, *viz*; A and Set B. The predation on I instar larvae was higher than on II, III and IV instars. However, there was a minor change in the predatory potential during the later part of the day, by feeding more on II, III and IV instars.

Table 1: Impact of prey density on the predatory potential of 12th instar *Bradinopyga geminata* recorded at three hours interval for Set A and Set B

Time of recording (h)	Average consumption (±SE) SET A (200:1)				Average consumption (±SE) SET B (1000:5)			
	I	II	III	IV	I	II	III	IV
9	73 (3.5)	51 (4.1)	36 (2.3)	29 (0.9)	387 (4.1)	265 (2.0)	184 (5.2)	154 (4.9)
12	18 (2.0)	7 (1.8)	3 (1.2)	3 (0.9)	53 (7.9)	59 (2.3)	32 (1.5)	19 (2.3)
15	10 (2.1)	6 (2.1)	4 (2.1)	3 (1.5)	37 (4.8)	22 (5.7)	25 (5.1)	11 (4.9)
18	29 (1.5)	24 (2.0)	21 (0.9)	17 (2.3)	115 (4.6)	136 (8.8)	87 (13.7)	71 (16.3)
21	17 (2.4)	15 (1.7)	19 (2.1)	17 (2.2)	54 (2.3)	69 (4.7)	75 (6.4)	71 (11.3)
24	7 (2.1)	7 (2.0)	9 (2.0)	9 (2.1)	28 (2.5)	37 (5.0)	27 (1.8)	21 (4.4)
3	33 (6.8)	54 (4.2)	34 (2.7)	30 (1.5)	127 (6.1)	126 (4.3)	205 (4.8)	191 (0.3)
6	10 (2.9)	13 (2.3)	8 (2.6)	8 (0.6)	86 (4.9)	133 (14.9)	111 (8.7)	91 (6.8)

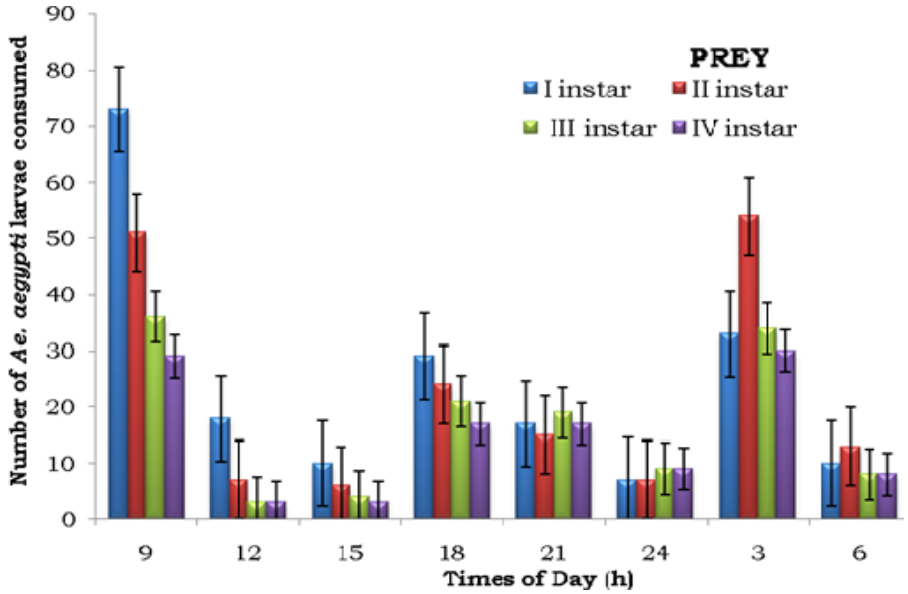

Fig. 1: Impact of prey density on the predatory potential of 12th instar *Bradinopyga geminata* recorded at three hours interval (200 larvae / 1 predator)

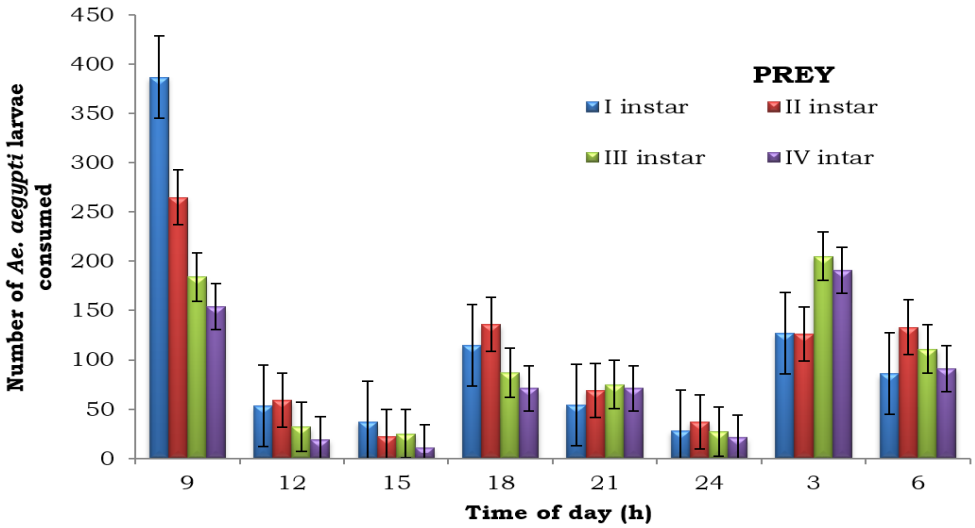

Fig.2: Impact of prey density on the predatory potential of 12th instar *Bradinopyga geminata* recorded at three hours interval (1000 larvae / 5 predators)

Cumulative number of prey for Set A *i.e.*, I instar larvae of *Ae. aegypti* consumed by a single 12th instar of *B. geminata* was 197, while 178, 133, 117 for II, III and IV instars respectively for 24 hours observation and Set B, I instar larvae of *Ae. aegypti* consumed by five 12th instar of *B. geminata* was 886. It was 846, 745, 629 for II, III and IV instars respectively for 24 hours observation. These results showed that the prey consumption by the predator was more on I instar larvae than other instars. The trend of prey consumption by the predator during the day was worked out by cumulative numbers (Figs. 3 & 4). In both cases, similar trend was observed, the consumption of I instar larvae was higher than the consumption of II, III and IV instars. The consumption of IV instar larvae was less, but the consumption of II, III and IV instar larvae was medium. This clearly demonstrates the preference of predator for the first instar larvae.

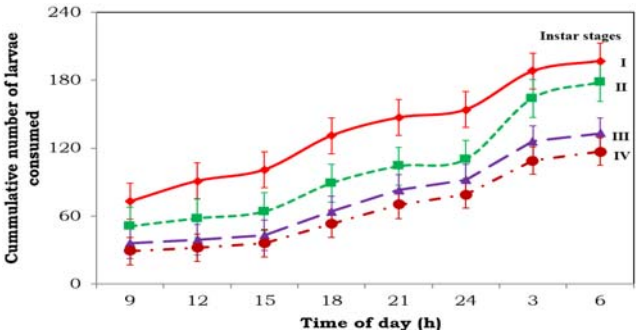

Fig. 3: Cumulative number of *Aedes aegypti* larvae consumed by 12th instar of *Bradinopyga geminata* over a period of 24 hours (predator – prey ratio was 1:200)

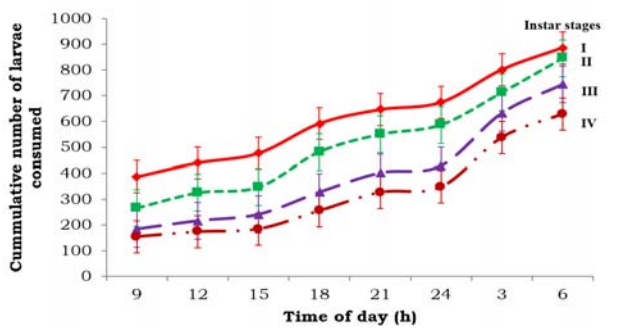

Fig.4. Cumulative number of *Aedes aegypti* larvae consumed by 12th instar of *Bradinopyga geminata* over a period of 24 hours (predator – prey ratio was 5:1000)

Single 12th instar *B. geminata* consumed 131, 89, 64 and 53 of I, II, III and IV instar larvae of *Ae. aegypti* respectively in day hours (6-18 hours) and 68, 89, 70 and 64 in night hours (18-6 hours) respectively. During day hours, single 12th instar of *B. geminata* consumed 46% and 54% of mosquito larvae in night hours for the prey density of 200 *Ae. aegypti* larvae. Five *B. geminata* consumed 592, 481, 327 and 255 of I, II, III and IV instars larvae of *Ae. aegypti* respectively in day hours (6-18 hours) and 295, 365, 417 and 374 in night hours (18-6 hours) respectively. During day hours, five 12th instar of *B. geminata* consumed 47%, while 53% in night hours for the prey density of 1000 *Ae. aegypti* larvae (Table 2). The pattern showed that *B. geminata* consumed more or less similar number of prey both during day and night hours, indicating that feeding occurred throughout.

Table 2: Predation of 12th instar *Bradinopyga geminata* on *Aedes aegypti* larvae during 24 hours period

Instars	SET A (200:1)		SET B (1000:5)	
	Day hours 6-18 (h)	Night hours 18-6 (h)	Day hours 6-18 (h)	Night hours 18-6 (h)
I	131	68	592	295
II	89	89	481	365
III	64	70	327	417
IV	53	64	255	374

In Set A, the average consumption of first instar *Ae. aegypti* was 197out of 200, with a daily feeding rate of larvae / predator in a 24 hour period. The percentage consumption was 98.9 for I instar, while the consumption of II, III and IV instars of *Ae. aegypti* was 178, 133, 117 respectively. The daily feeding rate of larvae / predator during a 24 hour period was 89, 66.7, 58.2 respectively. In Set B, average consumption of first instar *Ae. aegypti* by five *B. geminata* larvae was 886 larvae out of 1000 in a 24 hour period. The percentage consumption was 88.6 for I instar, while for the II, III and IV instars of *Ae. aegypti* it was 846, 744, 629 respectively in a 24 hour period, and the percentage consumption was 84.6, 74.4, 62.9 respectively (Table 3).

Table 3: Predatory efficacy of 12th instar of *Bradinopyga geminate*

Larval instars of *Ae. Aegypti*	Predator consumption		Clearance rate (CR)		Predatory Impact (PI)	
	Set A	Set B	Set A	Set B	Set A	Set B
I instar	98.9	88.6	22.97	22.42	4.12	3.69
II instar	89.0	84.6	22.44	22.19	3.71	3.53
III instar	66.7	74.4	21.00	21.55	2.78	3.10
IV instar	58.2	62.9	20.32	20.71	2.42	2.62

The prey consumption by the predator on the I instar larvae was maximum, while it was minimum on the IV instar. The consumption was medium for the II and III instars of the prey. This was similar in both A and B sets of experiment. This confirms the predator's preference for the I instars of the prey. The predatory impact (PI) of both sets A and B was highly significant for the first instars, as the prey size was small and is easy to capture. The number of prey killed varied with the density of preys and predators available in a volume of water. Comparative accounts of clearance rate of both sets A and B was highly significant for the first instar *(P<0.01)*.

The maximum PI value was observed for the first instar of *Ae. aegypti*, in both A and B set of experiments. The PI values for I instar in A and B was 4.12± 0.05 and 3.69±0.02 respectively and the t– values were significant *(P<0.01)*. Predatory impact was observed for the II, III and IV in both A and B sets of experiment and the t-value was not significant *(P>0.05)* (Table 4).The maximum clearance rate value was observed for the I instar of *Ae. aegypti*, when the prey size was smaller, while the CR was higher in both A and B set of experiments. The CR value for the first instar in both A and B was 22.97± 0.06 and 22.42±0.03 and the t– values were significant *(P<0.01)*. Clearance rate observed for the II, III and IV in both A and B was not significant (*P>0.05)* (Table 5).

Table 4: Predatory Impact (PI) of *Bradinopyga geminata* (n=3 replicates) against *Aedes aegypti* (I-IV) instars larvae

Species	Instars			
	I	II	III	IV
SET A	4.02 - 4.17 4.12 ± 0.05	3.48 - 4.04 3.71 ± 0.17	2.65 - 2.92 2.78 ± 0.08	2.33 - 2.52 2.42 ± 0.05
SET B	3.67 - 3.73 3.69 ± 0.02	3.41 - 3.62 3.53 ± 0.06	3.09 - 3.11 3.10 ± 0.01	2.59 - 2.65 2.62 ± 0.02
t-Test	*P < 0.05**	*P > 0.05*	*P > 0.05*	*P > 0.05*

*Values are in range, mean ± S.E. in larvae/day/predator; * Significant at 5% level (P<0.05)*

Table 5: Clearance rate (CR) of *Bradinopyga geminata* larvae (n=3 replicates) against *Aedes aegypti* (I-IV) larvae

Species	Instars			
	I	II	III	IV
SET A	22.85 - 23.03 22.97 ± 0.06	22.12 - 22.87 22.43 ± 0.23	20.76 - 21.24 20.99 ± 0.14	20.13 - 20.51 20.31 ± 0.11
SET B	22.39 - 22.48 22.42 ± 0.03	22.02 - 22.32 22.19 ± 0.09	21.53 - 21.57 21.55 ± 0.01	20.64 - 20.77 20.71 ± 0.04
t-Test	*P < 0.05**	*P > 0.05*	*P > 0.05*	*P > 0.05*

*Values are in range, mean ± S.E. in larvae/day/predator; *Significant at 5% level*

5. Discussions

This study revealed that *Bradinopyga geminata* is an active feeder and able to consume mosquito larvae. The predatory impact, reflecting the prey killing capability of *B. geminata* is expectedly more compared to the first instar larvae of *Ae. aegypti*, owing to its size and energy requirements. Predator prefers the prey of first instar than the late instars due to its size and clearly demonstrated that the preference of predator over the first instar larvae of prey.

The predator impact is not much different when the number of preys and predators increased. Thus *B. geminata* consumed more *Ae. aegypti* larvae during the first hour of predation, which declined with increasing larval size and instar stage.

Tyagi and Venkatesh (2013) [50] developed an indigenous biocontrol method "Minimum Re-inforcement Method" (MRM) for suppressing the larval population of dengue vector, *Ae. aegypti*, albeit still at laboratory level, and revealed that single *B. geminata* larva is sufficient for eliminating the huge mass of larval mosquitoes breeding in a cement tank / cistern. Corbet (1980) [4] suggested that introducing the dragonfly larvae into each domestic water-storage containers at monthly intervals can result in virtual elimination of *Ae. aegypti* larvae within three weeks. Sebastian *et al.*, (1990) [9] suggested that the *Crocothemis servilla* was able to suppress *Ae. aegypti* larval and adult population for about 6 weeks.

Bradinopyga geminata can oviposit in the ornamental cement tanks, overhead tanks and garden ponds [51]. The species employed in our experiment were also collected from the ornamental cement pond. Kumar (1973) [45] showed that the appearance of *B. geminata* larvae in metal drums were in brownish to blackish cylindrical iron barrels and were unusual in its seasonal distribution in north-western India. Hearle (1926) [52] pointed out that the value of dragonfly nymphs as mosquito enemies is limited by their bottom-feeding habits. Venkatesh and Tyagi (2013a) [17] concluded that *B. geminata* and *Ceriogrian coromandelianum* are effective as predators of dengue vector *Ae. aegypti*, due to its large size and *B. geminata* which almost invariably breeds in cement tanks and expected to consume good number of mosquito larvae and be effectively used as a biocontrol agent for the control of dengue. The major requirement of a program that will help stop the transmission of mosquito-borne diseases is the ability of the control agent to adapt to various water bodies that are scattered within and around human settlements where vectorially important mosquitoes predominantly breed. Kumar *et al.*, (2008) [53] concluded that the mosquito larval rate decreased with increasing larval size and instar stage. They suggested that the feasibility of using copepods in large-scale control programmes.

Chatterjee *et al.*, (2007) [12] found a significant decrease in *Anopheles subpictus* larval density in dipper samples, 15 days after the introduction of *Brachytron pratense* dragonfly larvae (10 individuals) in concrete tanks under field conditions in India. He demonstrated the biocontrol efficacy of aquatic larvae of the *Brachytron pratense* dragonfly against the larvae of the mosquito *An. subpictus*. Aditya *et al.*, (2006) [46] suggested that in comparison to *Toxorhynchites splendens*, the *Rhantus sikkimensis* beetle was much more efficient as predator, the predation rate as well as clearance rate. Kumar *et al.*, (2008) [53] evaluated the larvivorous efficacy of three predators viz., mosquitofish, dragonfly naiads and copepods, and concluded that the predation rate on mosquito larvae decreased with increasing larval size and instar stage. However, Bay (1974) [54] reported that dragonfly larvae are known to prey heavily on bottom feeding mosquitoes like *Aedes* larvae and also suggested that most mosquito larvae are easy to rear and maintain, and make excellent prey for a wide variety of aquatic organisms. *Ceriagrion coromandelianum* and *Brachydiplax chalybea chalybea* prey consumption varied significantly with the prey and predator densities for both the Odonate predators [17].

Sebastian *et al.* (1980) [8]. found and concluded his study to complete elimination of all *Aedes aegypti* larvae and pupae between day 4 and 9 depending on the density of aquatic stages of mosquitoes present per container when dragonfly larva, *C. servilia* was used. In West Bengal, Mandal *et al.*, (2008) [13]. suggested that the larvae of five Odonate species used in semifield conditions, significantly lowered the mosquito larval density after 15 days of introduction. However, zygopteran larvae tend to occur in greater numbers than anisopteran larvae if such a cement tank is harbouring vegetation [17].

From the results it was evident that the predator, *B. geminata* can consume a good number of larvae of *Aedes aegypti*, though considerable difference in the number of predator with number of preys. The predatory impact, reflecting the prey killing capability of *B. geminata* is expectedly more compared to the first instar larvae of *Ae. aegypti*, owing to its size and

energy requirements. When the predation rates are considered in respect to the prey size and density, the rate of consumption of *B. geminata* reflects its ability to kill more smaller preys (I instar of *Ae. aegypti*). This proportionate killing was reduced gradually in the II, III and IV revealing its lower ability to kill target prey to *B. geminata*. Also, the body-sizes of the preys are different and thus, *B. geminata* had a greater predatory impact and highly significant to the first instar larvae of *Ae. aegypti* to sustain its feeding requirements. However, certain general rules guide the pattern of arthropod predation related to body size, prey density and other factors pertaining to the biology of predators [55-57].

This study concluded that the prey density had an impact on the predatory potential of 12th instar of *B. geminata* and this was observed by exposing two different prey-predator combinations (Prey: Predator; 200:1 and 1000:5). In these two cases, both the number of prey and predator was increased in a proportionate manner. In both cases, the preference seems to be similar, the consumption of 1st instar larvae of *Ae. aegypti* was more in both cases and the potential was also more or less similar. The results of this study indicate that this proportion of prey-predator seems to be optimum. The consumption during 24 hour period was similar in these two combinations. The percentage of consumption was similar both during the day and night hours of the day and therefore there was no marked variation in the predatory potential of *B. geminata*. The prey potential behaviour was also confirmed by the estimation of clearance rates, and the results were statistically significant. This predator needs to be tested under field conditions in order to promote them for regulation of dengue vector mosquitoes during epidemic season.

6. Conclusion

From the results it is concluded that the predator of *B. geminata* can consume a good number of larvae of *Ae. aegypti*, though considerable difference in the number of predator with number of preys. When the predation rates are considered in respect to the prey size and density, the rate of consumption rate in *B. geminata* reflecting its ability to kill more smaller preys (I instar). This proportionate killing was reduced gradually in the II, III and IV revealing its ability to kill target prey to *B. geminata*. Also, the body-sizes of the preys are different and thus, *B. geminata* has a greater predator impact and highly significant to the first instar larvae of *Ae. aegypti* to sustain its feeding requirements. This study clearly demonstrated the preference of predator over the first instar larvae of prey.

7. Acknowledgements

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