

## Original Research Article

# A Study on the Predatory Potency of Dragonfly, *Bradinopyga geminata* Nymphs over the Immature Stages of the Filarial Vector, *Culex quinquefasciatus* Say

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## ABSTRACT

Pressure of urbanization, improper management of the domestic, polluted and other neglected water collections has led to the formation of enormous mosquito breeding sites resulting in the spread of various mosquito borne diseases such as Japanese encephalitis, chikungunya, dengue, filariasis etc. Since many decades chemical insecticides are widely used to control mosquitoes. Residues of these insecticides are found in the air, water, soil and even within the body of organisms. The use of chemical insecticide is now no more the concrete solution to suppress the vector population. Mosquitoes have many natural enemies, both as predators and parasites. Certain species of fishes, birds, bats, and insects prey upon them. Dragonflies are one among these predatory insects belonging to the order *Odonata*. In the present study an attempt has been made to understand the predatory potency of the locally available dragonfly, *Bradinopyga geminata* nymphs over the immature stages of the mosquito, *Cx. quinquefasciatus*, with the aim to develop an eco-friendly, economical and safe alternative in mosquito control. Results indicate that the maximum predation was on the first instar larvae while the minimum predation was of the pupal stage by different sizes of nymphs. The present study reveals voracious, intense and active predation of dragonfly nymphs over the *Cx. quinquefasciatus* immature larval and pupal stages.

## Keywords

*Culex quinquefasciatus*,  
Dragonfly nymphs,  
*Bradinopyga geminata*,  
Predation

## Introduction

Mosquitoes pose a major threat to human health as they play a predominant role in the transmission of Japanese encephalitis, filariasis, chikungunya, malaria, dengue and other such vector borne communicable diseases (Jang *et al.*, 2002). Among these communicable diseases, Lymphatic filariasis caused by *Wuchereria bancrofti*

is a major problem of the tropics and subtropics. According to the expert committee on filariasis, 905 million people were at risk of filariasis with 90.2 million of victims worldwide in 1984 and the figures were 751.4 million and 78.6 million in 1992 respectively (Ottesan *et al.*, 1997). At present, 1.3 billion people

are at risk of lymphatic filariasis infection and about 120 million people are affected in 83 countries amongst them about 45.5 million people are from Indian subcontinent (WHO, 2006). It is an alarming situation to limit filarial transmission through different means by reducing vector population by larvicides, adulticides and introduction of natural predators of mosquitoes in ecosystem. Japanese encephalitis is another major mosquito borne disease endemic in at least 21 countries with nearly 30,000-50,000 cases annually from Asia (Solomon, 2006). In India, as estimated 378 million people are living at the risk of Japanese encephalitis in 12 states including Uttar Pradesh (Okuno, 1978; Bharadwaj, 1981). The chronic forms of these diseases are assumed to be debilitating, leading to the restriction in the economic productivity (Evans *et al.*, 1993).

The mosquito *Culex quinquefasciatus* Say, is the major vector of Filariasis, Japanese encephalitis and other plagues throughout the world, especially in the tropical and the subtropical countries (WHO, 2006). It is found throughout all the warmer parts of the world breeding in association with human habitation, preferring polluted waters such as sewage and silage water collections, including cesspools, cesspits, drains, septic tanks and the places provided with a sufficient degree of organic pollution. It is claimed that the careful and prolonged control of the mosquito, *Culex quinquefasciatus* can eradicate filariasis, Japanese encephalitis and other such mosquito borne diseases.

For many decades chemical insecticides are widely used to control mosquitoes. They are generally chlorinated hydrocarbons like DDT, Dieldrin, endosulphan; organophosphates like

diazinon, bensolid and fenthion and carbamates like adiazarb, carbofuran and carboxyl. Residues of many such insecticides were found in the air, water, soil and even within the body of organisms. Some of these get incorporated within the human bodies as persistent deposits in fatty tissues and sometimes even found being secreted in the mother's milk. The use of chemical insecticide is now no more the concrete solution to suppress the vector population. Biological control methods particularly the application of the predators like indigenous larvivorous fishes, dragonfly nymph, bugs, and parasites like bacteria, fungi, nematodes etc., provide an alternative means of mosquito control. The distinct advantage with these biocontrol agents are their ability to kill target species at low doses, safety to non-target organisms, easy application in the field, inexpensive production, lack of infectivity and no pathogenicity in mammals including man.

Mosquitoes have many natural enemies, both as predators and parasites. Certain species of birds, bats, and insects prey upon them. Many species of fish feed on their larvae and certain top feeding minnows, particularly of the genus *Gambusia*, have been extensively employed in many parts of the world in mosquito control (Das and Prasad, 1991). Invertebrate predators have been shown to be responsible for the drastic reduction in the larval population of mosquitoes. Important group of insects predators in the rice field includes *Coleopterans* specially *Dysticidae* and *Hydrophilidae*, *Hemiptera* particularly *Notonectidae* and *Odonata* mainly *Anisopterans*. Predators usually have a limited breeding season and longer generation time than that of mosquitoes and hence they play a vital

role in control of mosquito larvae. Among these biological agents of mosquito control the *Odonates* are one of the best predators. The importance of Dragonflies (*Odonata*) as consumers of mosquitoes has been known for a long time ago (Corbet, 1980).

*Odonates* were one of the first arthropods to be examined as biological agents to control mosquitoes. They are voracious and important predators of mosquito larvae in the fresh water ecosystems. They detect their prey by compound eyes and mechanical receptors and capture them with their labium. Both the nymph as well as the adults catches mosquitoes (Corbet, 1980).

In feeding experiments, *Anisopteran* nymphs proved to be extremely voracious. Nymphs of *Aeshnacyanea* consumed up to 100 mosquitoes larvae each day and approximately around 2110-2120 during its life time (Becker *et al.*, 2003). Such research finding indicates that the development, survival and abundance of larval mosquito populations in the field are limited by predaceous insects which are primarily responsible for mortality in immature stages of mosquitoes.

This effect has been reported in many different aquatic habitats and is responsible for restraining the density of such prey populations below the critical threshold where transmission of diseases could not occur (Das *et al.*, 2006).

Today, environmental safety is considered to be of paramount importance. Therefore the present study was undertaken to study the predatory potency of the locally available nymphs of dragonfly, *Bradinopyga geminata* over the immature stages of the mosquito, *Culex quinquefasciatus* Say.

## Materials and Methods

### Collection of *Culex quinquefasciatus*

**Say:** The larvae and adults of the mosquito, *Culex quinquefasciatus* were collected from various mosquito-genic regions of Agra city (26° 44' N to 27° 25' N latitude; 77° 26' E to 78° 32' E longitude). Sampling of larvae and pupae were done using aquatic handdipnet having sieve with fine pores and a diameter of 4 inch. The larvae and pupae were identified, numbered and carried to laboratory along with water samples in specimen bags made of polythene, keeping half of the space in the bag empty for aeration. Sometimes pipette and ladles were also used for collection. Egg rafts, if any, were collected in the filter paper and kept in the specimen box. The samples were marked carefully recording the date, location, type of site and other physical parameters. Identification of species was done following standard key (Barraud, 1934). Fourth instar larvae were taken for categorization and reared until adult emergence for further confirmation of the species. Both the larvae and adults were identified and preserved.

### Collection of dragonfly nymph

Dragonfly nymphs were collected from the various region of Agra city. Nymphs were collected with the help of the dipper and strainer from the ponds, ditches, cemented tanks and other water bodies. The collection was done from the water bodies by sweeping the aquatic handdipnet from a depth of one to two feet inside the water in the downward to upwards direction. Nymphs were numbered, recorded and then transferred to the sampling jars along with the leaf litter, water weeds, with very little free water. These sampling jars were then kept in the sampling bags and

transported carefully to the laboratory. These nymphs were identified at School of Entomology, Department of Zoology, St. John's College, Agra using standard keys (Fraser, 1934).

### **Laboratory rearing of *Culex quinquefasciatus* Say**

The colony was maintained from the egg rafts. The egg rafts were kept in white enamelled breeding bowls 10 inches in diameter, 5 inches deep and half filled with de-chlorinated tap water. The water in the breeding bowls, on the onset of the first instars larvae, was supplemented with yeast at an interval of every twenty four hour, till the larvae transformed into pupae. Each bowl contained about 150-200 larvae. The amount of food added daily was increased with larval stage. The scum, if formed, was removed using a glass rod. The duration of larval stage was seven to eight days at  $28\pm 1^{\circ}\text{C}$  and about 65 – 80 percent of relative humidity. Pupae were collected daily and transferred in groups of fifty pupae at a time into beakers; half filled with clean water and placed in empty 18"×18"×18" mosquito breeding cages. The metamorphosed adults were fed with 10 % sugar solution or honey soaked in a piece of cotton for three to four days. Each cage contained about 200♀ and 100♂ adults which were allowed to mate.

The females were given blood meals from a healthy but immobilized pigeon which was placed inside the rearing cages during nights on alternate days. For egg laying suitable containers half filled with water were introduced inside the breeding cages which contained fertilized females. Each container was lined with filter paper to prevent the egg rafts from adhering to the containers walls. The egg rafts were

processed again to yield adults as described above. Deposited egg rafts were collected daily, numbered, marked and incubated for experimental usage.

“Rekha”, a chemical repellent was used to mark the boundary of the rearing cages so as to check the entry of ants, spiders and other such insects which may damage the mosquito colony.

### **Laboratory rearing of dragonfly nymphs**

The collected nymphs of the dragonfly, *Bradinopyga geminata*, were released in the aquarium with dimension of 60 × 30 × 35cm and the water was filled up to 10 cm for maintaining them alive. This water contained both protozoan and planktonic algae, which are a natural source of food for dragonfly. Once they are measured, each dragonfly nymphs were given their own containers and fed with bloodworms or mosquito larvae. The nymphs were fed twice or thrice a week during winters and several times a week in summers. When the nymphs were about to emerge as adults or imago, they were transferred into emergence cages, allowed to emerge, and then released back to the environment. A few adult specimens were kept in glassine envelope after killing and preserving using acetone in School of Entomology, Department of Zoology, St. John's College, Agra for further reference.

### **Predation experiment**

To determine the predatory potency of the locally available dragonfly, *Bradinopyga geminata* nymphs the method followed by Singh and Dhiman (2003) was used with slight modifications. The dragonfly nymphs, of different sizes were brought from the field and maintained in the

aquarium. Three stages of dragonfly nymphs of different size i.e. small (5mm), medium (10 mm) and large (15mm), were measured lengthwise and used in predation experiments. All three nymphal stages of *B.geminata* were provided with *Cx. quinquefasciatus* larvae as supplementary food, obtained through laboratory rearing. A series of experiments were conducted in the laboratory within the troughs of 500 ml capacity containing 300 ml of water with larval food. One hundred larvae of each instars and pupae were provided to each size of dragonfly nymphs i.e. small, medium and large, in five replicates along with control. Thus each size of dragonfly nymph was studied with five replicates each, separately for predation against every immature stages of the mosquito, *Cx. quinquefasciatus*. Observations on prey mortality were made by counting and recording of live mosquito larvae and pupae present in the trough at an interval of 12 hours, 24 hours and 36 hours.

## Results and Discussion

Results of the feeding rate of dragonfly nymph on the immature stages of the mosquito, *Culex quinquefasciatus* indicates that the maximum predation was on the first instar larvae while the minimum predation was of the pupal stage by different sizes of nymphs (small, medium and large) Table 1, 2 & 3. Increase in immature stages of *Cx. quinquefasciatus* resulted in the decreased feeding rate of nymphs. As the size of the nymphs increased, there was increase in predation rate also. It is shown in the experiment that the nymphal stages (small, medium & large) seemed to prefer early larval stages of mosquito (Figure 1, 2 & 3). Pupae were least preferred by all nymphal size (Table 1, 2 & 3). It was also observed that the large sized nymphs predated more larvae of *Cx.*

*quinquefasciatus* without showing any preference for first or fourth instar larvae while smaller nymphs preferably predated over initial stages of the *Cx. quinquefasciatus*. The results also indicates that among the different nymphal size (small, medium and large) the small sized nymphs (5mm) highly predated over the third instar larvae 39.1, 52.3 and 68.4 after 12 hours, 24 hours and 36 hours respectively (Table: 1, 2 and 3 ). There was no marked difference in the predation by medium (10 mm) and large (5 mm) nymphal size of dragonfly. However, both large and medium size of dragonfly nymphs preferred early stages of *Cx. quinquefasciatus* larvae (Figure: 1, 2 and 3). Pre-moulting time of nymphal stage also decreased the feeding rate.

Introduction of dragonfly nymphs as predators over mosquito larvae in water reservoirs has resulted in reduction of mosquito larvae and even their complete elimination in the water bodies (Sebastian *et al.*, 1980; Sebastian *et al.*, 1990; Garcia *et al.*, 1996). The present study reveals voracious, intense and active predation of dragonfly nymphs over the *Cx. quinquefasciatus* immature larval and pupal stages. From the results it is clear that the mosquito larvae are the very apparent food of dragonfly nymphs. The results indicate that dragonfly nymphs preferred mostly the first instar larvae (Figure 1, 2 and 3). Results of feeding rates of dragonfly nymph, *B. contaminata* over the aquatic stages of *Anopheles stephensi*, and *Aedes aegypti* also indicated that the maximum predation was of first instar larvae (127.8 and 112.5 respectively) while the minimum was of pupal stage (Singh and Dhiman, 2003). The dragonfly nymphs belonging to the *Labellula spp.* in an experiment over the predation of *Aedes aegypti* larval and pupal stages also

reported that the dragonfly consumed both the stages with preference towards the smaller once over the pupae (Alahmed *et al.*, 2009). Rashed *et al.*, (2005) also reported that the dragonfly preferred small prey over large prey in his experiments first using small *H. rapax* and large *Sarcophaga spp.* The size of prey in relation to the predator is important factor influencing the predation rate by nymph. The reason of decreasing predation with increase in size of immature stages of *Cx. quinquefasciatus* is due to increase in size of prey, which provides more quantity of food intake resulting in reduction in number of prey to be preyed. However, pupae were preferred least over all nymphal stages. Lee (1967), found that the mosquito larvae are consumed more than pupae by predator due to inclination of pupae to exhibit rapid tumbling action when startled.

The present study also suggests that the rate of predation increases along with the size of dragonfly nymphs and the decrease in feeding rate with increase in larval stages of mosquito (Fig. 1, 2, and 3; Table. 1, 2 and 3).

In a study of predation by dragonfly nymph, *Orthemis spp.* over the mosquito larvae, it was revealed that the large nymphs ate more mosquito larvae without showing any preference for the immature stages of mosquito (Lee, 1967; Cordoba and Lee, 1995). During a study on the role of dragonfly nymph *Brachytron pratense*, as a bio control agent of mosquito larvae, it was noted that the small sized nymphs were not interested to prey upon fourth instar stage of mosquito larvae in laboratory; however on starvation they consumed early instar larvae of mosquito (Chatterjee *et al.*, 2007). The food habitats of nymphs change with advancement of

age and when they are advanced in age they are particularly addicted to the *Culicidae* larvae. The results of the present study also indicates that among the different nymphal size i.e. small, medium and large, the small sized nymph (5mm) mostly predated over the third instar larvae of *Cx. quinquefasciatus*, 39.1, 52.3 and 68.4 after 12 hours, 24 hours and 36 hours respectively ( Table. 1, 2 and 3).

Mandalet.al. (2008), in his experiment related to the predation of dragonfly nymph over *Cx. quinquefasciatus*, reported that the feeding rate varied significantly between dark and light conditions and concluded that darkness had negative influence on the predatory potency of dragonfly nymph. Odonata nymphs belonging to *Tramea spp.* consumed more larvae in darkness than in normal illumination and it was noticed that darkness did not affect the predatory activity of the nymph (Lee, 1967).

In the present study also, there was no marked differences in predation by different size of dragonfly nymph in 12, 24 and 36 hours, however both the medium and large sized nymphs seems to be predated more over the first and second instar larvae of *Cx. quinquefasciatus*.

The other factors that determine the predatory potency of dragonfly nymphs is the predator or prey density. It was believed that the prey consumption was inversely related with the space. The predation of dragonfly nymph, *Enallagma civile* over *Cx. tarsalis* showed that when the density of prey and predators were varied more prey were consumed as prey density increased however prey were consumed at higher predator densities (Miura and Takahashi, 1988).

**Table.1** Predatory potency of *Bradinopyga geminata* nymphs over the mosquito larvae and pupae of *Culex quinquefasciatus* Say. in 12 hours

Nymphal Size	Larval instars and Pupal stage				
	I	II	III	IV	P
	SD	SD	SD	SD	SD
Small (5mm)	24.1±3.8	30±5.2	39.1±4.1	11.7±2.2	5.3±2.1
Medium (10mm)	51.6±4.7	41.2±4.9	32.7±3.7	21.3±4.0	20.1±1.8
Large (15mm)	49.3±2.8	47.1±3.9	44.8±5.3	41.5±3.4	22.3±2.7

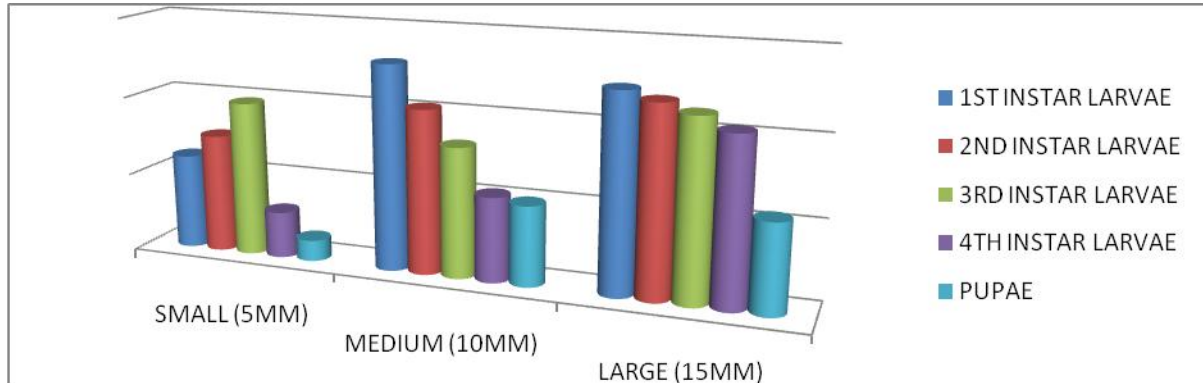
**Table.2** Predatory potency of *Bradinopyga geminata* nymphs over the mosquito larvae and pupae of *Culex quinquefasciatus* Say. in 24 hours

Nymphal Size	Larval instars and Pupal stage				
	I	II	III	IV	P
	SD	SD	SD	SD	SD
Small (5mm)	33±4.35	48.6±3.0	52.3±6.11	16±3.6	8±1
Medium (10mm)	72±2.64	52±2.6	41±3.6	29±6.55	27±3
Large (15mm)	79.6±6.65	65.6±3.78	68±3	57±5	31.3±2.5

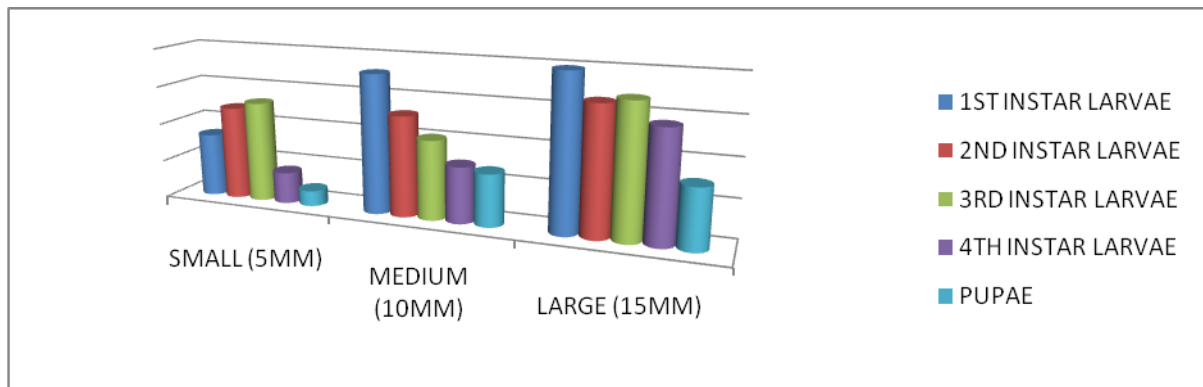
**Table.3** Predatory potency of *Bradinopyga geminata* nymphs over the mosquito larvae and pupae of *Culex quinquefasciatus* Say. in 36 hours

Nymphal Size	Larval instars and Pupal stage				
	I	II	III	IV	P
	SD	SD	SD	SD	SD
Small (5mm)	51.9±3.9	60.7±4.6	68.41±5.1	29.8±5.8	19.6±3.5
Medium (10mm)	93.8±3.2	86.4±5.0	77.4±4.8	69.5±6.3	33.1±4.9
Large (15mm)	92.21±3.8	90.3±3.7	91.7±3.8	73.0±6.1	39.8±4.7

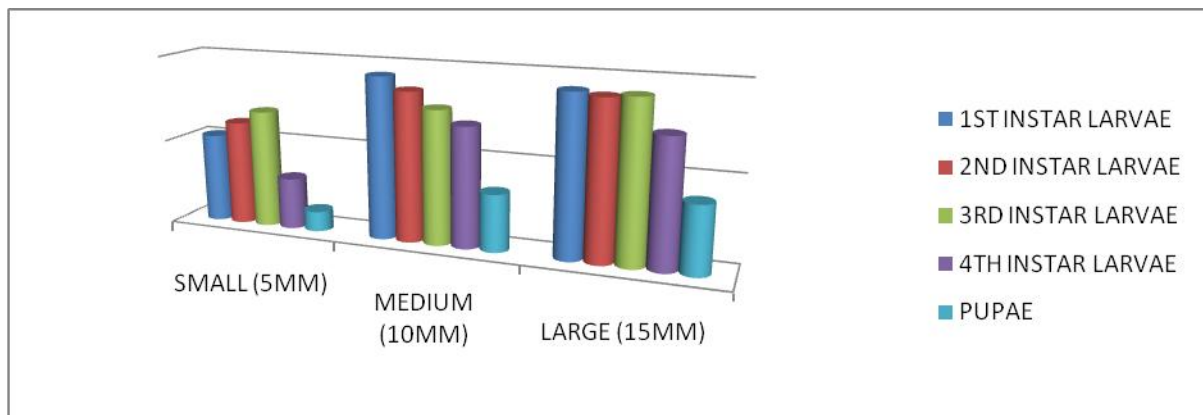
**Figure.1** Predatory potency of *Bradinopyga geminata* nymphs over the mosquito larvae and pupae of *Culex quinquefasciatus* Say. in 12 hours



**Figure.2** Predatory potency of *Bradinopyga geminata* nymphs over the mosquito larvae and pupae of *Culex quinquefasciatus* Say. in 24 hours



**Figure.3** Predatory potency of *Bradinopyga geminata* nymphs over the mosquito larvae and pupae of *Culex quinquefasciatus* Say. in 36 hours





Nymphs of five *Odonate* species *Aeshna flavifrons*, *Coenagrion kashmirum*, *Ischnura forcipata*, *Rhinocypha signipennis* and *Sympetrum durum* in semi-field conditions in West Bengal, India, significantly lowered the mosquito larval density in dipper samples after 15 days from the introduction, followed by a significant increase of larval mosquito density after 15 days from the withdrawal of the larvae (Mandal *et al.*, 2008). These results are suggestive of the use of *Odonata* larvae as potential biological agent in regulating the larval population of mosquito vectors. Mosquito ecologists have increasingly taken the approach that understanding how a predator, directly and indirectly, affects community structure is also important for understanding under what set of environmental conditions a predator will be effective in reducing mosquito populations. Stavet *al.* (1999) reported that the predaceous dragonfly larvae of *Anaximperator* produced 52% reduction in *Culiseta longiareolata* oviposition in outdoor artificial pools. The reduced number of *Cs. longiareolata* egg rafts found in the presence of *A. imperator* was largely due to oviposition habitat selection by *Cs. longiareolata* females. Stavet *al.* (2000) found that the egg rafts of the mosquito *Cs. longiareolata* deposited in the free *Anax* treatment were less than deposited in caged *Anax* and control treatments. There was statistically significant difference in the number of egg rafts between control and caged *Anax* pools which means that, while *Culiseta* females oviposit less egg rafts in the presence of *Anax*, they did not respond to predation risk from the caged *Anax*.

The concept of integrated control is a fairly specific one, which historically has meant the use of a combination of chemical and biological agents in as

compatible a manner as possible (Axtell, 1979). Sometimes cultural or physical control methods have been included. Predaceous insects are closely associated with mosquito immature as they co-inhabit in a wide variety of aquatic habitats such as rice fields, tree holes, man-made ponds, snowmelt pools, temporary lagoons, floodwaters and rain pools. These predators significantly affect the survival, development and recruitment levels of mosquitoes which most likely has an influence on vector-borne disease transmission rates. Biological and physical conditions were found to influence capacity of such predators. Biological conditions divided into predator and prey factors. Species, competence and predator prey density were the most common predator factors while species, stage and prey density were more likely prey factors. Illumination, temperature, container size and foraging area were the physical conditions that have been searched. Also field studies and implementation of predaceous aquatic insects in integrated vector control were documented in some circumstances. Dragonfly nymphs capture mosquito larvae by the modified labium, which is drawn out into a prehensile organ called the mask. While catching the prey, the mask is thrown forward and extended with incredible swiftness and the prey transfixed with the hook is drawn into the mouth cavity.

As can be expected, further studies are needed to ensure successful and satisfactory mosquito control with predaceous insects. Another important advantage of predators is their released kairomones that have the potency to repel ovipositing female mosquitoes for over a week. If these kairomones were commercially produced, they may provide eco-friendly and effective mosquito

control, but more research is necessary to determine their total impact. Thus, understanding the interaction between mosquito vectors and their aquatic predaceous insects is imperative for developing and implementing successful biological or integrated control measures that include the use of predators to control the vector population.

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