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Biocontrol efficiency of *Nepa cinerea* Linnaeus 1758 (Hemiptera: Nepidae) against the vectors of dengue and filarial fever

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Abstract
Mosquitoes are important vectors of several tropical diseases. Aquatic insects used as biocontrol agents for mosquitoes are given prime importance because of its ecofriendly nature. In the present study, the adults of *Nepa cinerea* were tested for its biocontrol efficiency against the instars of *Aedes aegypti, Aedes albopictus* and *Culex quinquefasciatus*. More preference was given to the III instar with regard to stage preference, and *Aedes aegypti* followed by *Aedes albopictus* and *Culex quinquefasciatus* with reference to the experimental vector species. The predatory impact and the clearance rate were high in *Aedes aegypti* followed by *Aedes albopictus* and *Culex quinquefasciatus* with values ranging from 14.05 to 15.70; 14.63 to 15.15; 14.83 to 15.05; and 2.64 to 2.71; 2.29 to 2.54; 1.43 to 2.17 per larvae per day predator respectively. Therefore, it can be concluded that *Nepa cinerea* adults could be used as an additional biocontrol agent against the vectors of dengue and filarial fever.

Keywords: *Nepa cinerea*, biocontrol, *Aedes aegypti, Aedes albopictus, Culex quinquefasciatus*

1. Introduction
Mosquitoes are looked upon not only as nuisance biters but also as vectors of important diseases, *viz*., malaria, lymphatic filariasis, Japanese Encephalitis, dengue, chikungunya and Zika virus fever particularly in the tropics [1, 2]. Many synthetic insecticides are widely used for controlling mosquito population but their harmful effects on non-target organisms and the development of physiological resistance in mosquitoes prompted to adapt biological control. Hence an outcry in exploring alternative, eco-friendly and simple sustainable methods of mosquito control is very essential. Various organisms known as natural biological control agents can be utilized to control mosquito populations especially the larval stages [3]. Thus avoiding the use of chemicals which harm the environment. In the field of applied ecology, it is desirable to use biological control agents that can adapt to mosquito breeding habitats as they pose no danger to the environment [4]. Usage of predatory insects supports sustenance of environmental network and biological integrity of the community, prey selectivity [5-7] and indirect interactions [6, 8] which are significant determinants of mosquito regulation. Yasuoka and Levins [9] suggested conservation of aquatic insects associated with mosquito larvae could be effective in controlling mosquito vectors, since both of them are aquatic in nature and therefore the efficient selection of effective natural enemies has become increasingly important for biological control program. *Nepa cinerea* a predaceous aquatic bug commonly called 'water scorpion' inhabits the littoral zones of ponds and temporary water bodies coexists with the mosquito larvae. The present study investigated the stage preference of *Nepa cinerea* against three mosquito species, *viz*., *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* and functional response of *Nepa cinerea* against *Aedes aegypti* with a view to optimize the biocontrol efficiency in addition to the estimation of the predatory impact as well as the clearance rate.

2. Materials and Methods
Adults of *Nepa cinerea* were collected from a pond in Mekkamandapam 20km away from Nagercoil, Tamil Nadu, India with the help of an insect net having 200µm mesh size. They were then brought to the laboratory and maintained in glass aquaria (30"x20"x20) containing

pond water. Few specimens of aquatic weeds like *Hydrilla* and *Eichornea*, and gravels were placed inside the aquarium to simulate natural conditions. The insects were collected seven days before the commencement of the experiments and were maintained in the laboratory for acclimatization with mosquito larvae as food. The larval instars of vector species, *viz*., *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* to be used in the experiments were kept in separate enamel trays.

2.1. Prey stage preference
Stage preference studies were conducted against all instars of the experimental vector species. To standardize the response, predators were starved for 24h in aquarium. The I instars of each experimental vector species were allowed to settle in three different containers and a single *Nepa cinerea* adult was introduced into each container. The same procedure was followed for II, III and IV instars also. Successfully preferred stage of the prey was noted to record functional response study.

2.2. Predatory impact
To a single adult *Nepa cinerea*, 50 III instars of each experimental vector species were offered as prey in a 500mL glass beaker separately for 24h and the number of instars consumed was noted. Further, the rate of predation was noted for three consecutive days, and the prey density was set to the same numbers after every 24h. The data obtained on predation were put to the following equation to calculate the predatory impact [10].

$$PI = \frac{PE}{T}$$

where,

PI: Predatory impact (Number of prey [larvae]/day)

PE: Number of prey killed/consumed

T: Time in days

2.3. Clearance rate
Three adults of *Nepa cinerea* were introduced to 100 III instars of each experimental vector species as prey in enamel tray (8"x5"x3") capacity with 1.5L of water, and the number of instars consumed after 24h was noted. For each adult, observations were made for three consecutive days using same individual (predator) and resetting the prey density (larvae) every 24h. The data obtained on the number of prey killed were calculated to estimate the clearance rate [11].

$$CR = \frac{V(InP)}{TN}$$

where,

CR: Clearance rate of predators (Number of prey [larvae] killed litres/days predator)

V: Volume of water

P: Number of prey killed

T: Time in days

N: Number of predators

Six replicates were maintained for each study. Data obtained on the number of prey killed were subjected to t-test to judge the difference in predation between the days as well as between the predator species [12]. PI and CR values were also compared through t-test.

3. Results
The predation of adult *Nepa cinerea* on the I, II, III and IV instars of *Aedes aegypti* larvae was 20.00 ±0.89, 22.00 ±1.29, 23.84 ± 1.06 and 21.17 ±2.27 respectively; and for *Aedes albopictus* it was 18.60 ±0.54, 19.40 ±1.51, 20.20 ±1.78 and 20.80 ±2.28 respectively; whereas for *Culex quinquefasciatus* it was 15.8 ±0.62, 17.2 ±1.92, 17.06 ±1.28 and 15 ±0.62 respectively (Figure 1). Further, *Nepa cinerea* preferred III larval instars with regard to stage preference. More predation efficiency was observed in *Aedes aegypti* followed by *Aedes albopictus* and *Culex quinquefasciatus*. The predatory impact values of *Nepa cinerea* against *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* ranged between 14.05 ±0.47 and 15.70 ±0.23; 14.63 ±0.38 and 15.17 ±0.71; and 14.83 ±0.23 and 15.05 ±0.47 respectively. T-test analysis showed significant result in all species except in *Aedes albopictus* on Day 3 (Figure 2). The clearance rate for *Nepa cinerea* against *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* ranged from 2.64 ±0.02 to 2.71 ±0.02; 2.29 ±0.40 to 2.54 ±0.19; and 1.43 ±0.18 to 2.17 ±0.10 prey larvae per day predator. T-test results indicated significance in all the three experimental vector species (Figure 2).

4. Discussion
Aquatic insects play a key role in mosquito management. Naturally occurring aquatic predators have been assumed as a significant ecological factor in regulating different mosquito species [13-17]. Biotic interactions such as competition and predation by aquatic predators have been reported to regulate the number of mosquito populations, thereby reducing the number of larvae. Aquatic coleopterans (notonectides and dysticids) and odonates have been observed to ingest mosquito larvae as a part of their natural food assemblages, and aquatic hemipterans (water bugs and back swimmers) have shown their tendency to feed on and regulate different mosquito species in aquatic habitats [18]. Therefore, selection of these biological control agents are to be based on their potential for unintended impacts, self-replicating capacity, climatic compatibility and their capability to maintain a very close interaction with target prey populations. Habitat specificity and their prey preferences act as an important criterion for selection of a potential biological control agent. Habitat specificity, functional response, prey preference and abundance in nature are important criteria for selection and qualification as a potential biological control agent. Amongst aquatic insects, *Nepa cinerea* has been documented for its mosquitocidal management [19] and they feed by piercing the prey with its rostrum, injecting digestive juices, and sucking the liquid contents from the prey. The present investigation gives a primary thought regarding the variation in the predation efficiency with change in the larval density and search area. This is a successful parameter in determination of actual feeding rate in field condition as it is conceivable to decide the quantity of predators that ought to be introduced in accordance to a specific prey density and available volume of search area in the field. Predator potentiality of coupled predators was particularly not as much as twice as that of an individual predator, which might be credited to intraspecific interventions such as mounting of one individual over another throughout the experiment.

In the present study, the frequency dependent mosquito larval size (I, II, III & IV) preferred by adult *Nepa cinerea* revealed the III instar of *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus*. Further, the study also highlighted that maximum feeding rate of *Nepa cinerea* was observed in III instar as I and II instars were smaller in size making it difficult for the predator in capturing the prey which were also similar to few studies which examined the effect of prey size on predator responses. Cogni *et al.* [20] reported that the small size reduviids preferred small size preys whereas large size predator preferred larger size prey. As a rule, it could be assumed that larger preys were easier to be detected by a predator which is corroborated in the present study. Predator growth rate depends on feeding rate which depends on the development stage of the predator that responds differently to variations to prey density. Growth rate of aquatic insect predator's increases with increased feeding rate as a function of prey density and feeding rate changes with instars development. Moreover, in the present study, the number of prey killed remained higher when three predators were present. The clearance rate reflected the combined effect of search ability, killing and consumption of prey by the predator and the prey invasion in unit time and space. Marin [21] reported that the predatory rate of *Diplonychus indicus* was higher against the dengue and filarial vector and a similar trend was also observed in the present study, with high predatory efficiency in *Aedes aegypti* followed by *Aedes albopictus* and *Culex quinquefasciatus* thereby enlightening the biocontrol efficiency of *Nepa cinerea* on mosquito larvae.

5. Conclusion

Vector control strategies especially biological control of mosquitoes by aquatic insects are widely accepted since these aquatic macro-invertebrate predators are reported to coexist abundantly with other organisms; including the aquatic stages of several species of mosquitoes. Though earlier workers have investigated the biological control potential of *Nepa cinerea*, a detailed account on their functional and numerical response against the variable of prey was portrayed in the present study as these parameters are needed before recommending their augmentative release in field conditions. Keeping in view of afore mentioned factors, *Nepa cinerea* proves to be successful candidate as an additional biocontrol tool in vector control programmes.

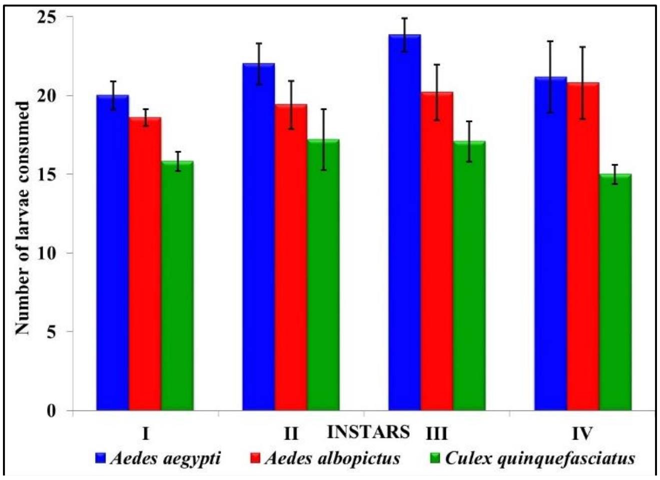

Fig 1: Prey stage preference of *Nepa cinerea*

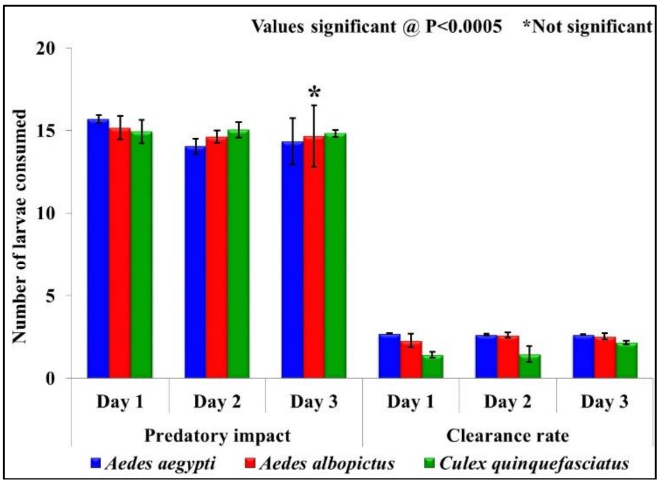

Fig 2: Predatory impact and Clearance rate of *Nepa cinerea* against III instar larvae

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