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Mosquito larvae specific predation by native cyclopoid copepod species, *Mesocylops aspericornis* (Daday, 1906)

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Abstract
Large sized cyclopoid copepods (having body size > 1.0 mm) act as predators of mosquito larvae which strongly influence the mosquito larval population. In the present study large sized native cyclopoid, *Mesocylops aspericornis* collected from local fish ponds of Ludhiana district of Punjab (India) was tested for its predatory potential against different types of mosquito larvae. Under laboratory conditions, *M. aspericornis* showed mosquito specific predatory behavior, as the predatory potential (number of larvae killed/cyclopoid/24hours) against 1st instar larvae of *Aedes* was observed to be maximum (24.27±3.95) followed by that of *Culex* (18.13±3.85) and least in case of *Anopheles* (0.13±0.09). This cyclopoid copepod species was also able to kill 2nd instar larvae of *Aedes* (14.19±6.25 larvae/cyclopoid/24hours) which was though less in comparison to its 1st instars. However, no predation of 2nd instar larvae of *Culex* and *Anopheles* and 3rd instar larvae of any of the three types of mosquito larvae was observed by this cyclopoid. Non-significant difference in predation of *Aedes* 1st instar larvae by *M. aspericornis* was observed during the trials conducted under simulated conditions performed in plastic cups, earthen pots and rubber tyres.

Keywords: *Aede*s, bio-control agents, cyclopoid copepods, mosquito larvae, predatory potential

1. Introduction
Mosquitoes are dangerous to public health as vectors that transmit a variety of disease causing agents which they acquire by biting an infected person. They are incredibly successful blood sucking insects and cause millions of deaths every year through the diseases they spread [1]. Some of the serious mosquito borne diseases are Malaria, Dengue, Chikungunya, Yellow Fever and Filariasis [2]. Considering the human health, it is essential to manage mosquito population, so as to control the occurrence of mosquito borne diseases. Several methods like trapping, chemical insecticides and bio-control agents are being used to prevent the spread of mosquitoes. Out of these, use of insecticides is a common practice being in operation nowadays for mosquito control. But, continuous use of these pesticides has generated chemical resistance among mosquitoes in addition to seriously harming the environment [3]. Therefore, search for effective mosquito control methods which are simple, cost effective and eco-friendly is the need of the hour. As a sustainable alternative, increased attention is being paid worldwide to biological means, which include the utilization of natural predators, pathogens and parasites [4, 5]. Like many predators in aquatic environments, cyclopoid copepods (a type of zooplankton) are known to feed voraciously on the 1st instar mosquito larvae [6, 7]. Mostly large species of cyclopoids like *Mesocyclops* and *Macrocyclops* (having body length > 1.0 mm) attack and consume newly hatched mosquito larvae without hesitation [8]. It is a well known fact that cyclopoid copepods thrive abundantly in most aquatic habitats. As native species are well adapted to local conditions, therefore, these local species of copepods must be explored to control the mosquitoes of that particular area. Thus, there is a need to search and identify the cyclopoid species co-existing with mosquito larvae in their breeding habitats and to understand their predatory behaviour with respect to mosquito larval population.

2. Materials and Methods
2.1 Collection and identification of cyclopoid copepods and mosquito larvae
Zooplankton were collected from fish ponds of Ludhiana district, Punjab (India) by using zooplankton net having mesh size 60 µm.

Large sized cyclopoid copepods (having body size > 1.00 mm) were extracted and identified up to species level on the basis of their morphological characters [9, 10] and the species level confirmation was done by Dr Ranga Reddy, Emeritus Professor (expert for zooplankton identification), Acharya Nagarjuna University, Andhra Pradesh (India). Single identified gravid female (having ovisacs filled with ova) was used as a start up culture and was fed on *Paramecium* for preparation of pure culture. For collection of mosquito larvae, water samples were taken from various standing water bodies, desert coolers, earthen pots and temporary water collections lying in peridomestic areas by using plastic dippers. The mosquito larvae were extracted manually and identified up to genus level under microscope on the basis of their morphological features by following the standard keys [11].

2.2 Testing the predatory potential of identified cyclopoid species against mosquito larvae

a) Under laboratory conditions

Identified large sized single adult cyclopoids were starved for 24 hours and introduced individually into petri plates having 100 ml of de-chlorinated water along with fifty 1st instar larvae of each type collected from the water samples. A control set (having equal number of larvae only as in experimental dishes and no cyclopoids) was also maintained simultaneously with each test trial. Ten replicates were run for each test and control sets. All petriplates were placed in an unilluminated B.O.D incubator maintained at temperature of 26±2 °C. Petri plates were checked after 24 hours and the number of larvae killed (either missing/consumed+damaged/dead) by each cyclopoid were recorded in all sets. Any missing larvae were considered as eaten by cyclopoids. Total mortality per petri plate was scored as per the formula given below:

$$\text{Larval mortality (\%)} = \frac{\text{Number of larvae missing/consumed} + \text{Number of larvae damaged /dead}}{\text{Total number of mosquito larvae taken initially}} \times 100$$

The same experiment was performed on 2nd and 3rd instars of different types of larvae as per the procedure given above.

b) Under simulated conditions:

As under laboratory conditions *M. aspericornis* showed efficient killing of *Aedes* larvae, therefore this experiment was performed with *Aedes* larvae only. For testing the predatory potential of cyclopoid copepods three types of containers (in triplicate) used were; plastic cups, earthen pots and rubber tyres. Water, soil, dried leaves and *Paramecium* culture were added in these containers to provide simulated conditions. Then, one cyclopoid and 50 *Aedes* 1st instar larvae were added in these test containers. A control set (having larvae only and no cyclopoid) was also run simultaneously (in triplicate) with each set. Mortality of mosquito larvae was observed after 24 and 48 hours (till the transformation of larvae into pupae).

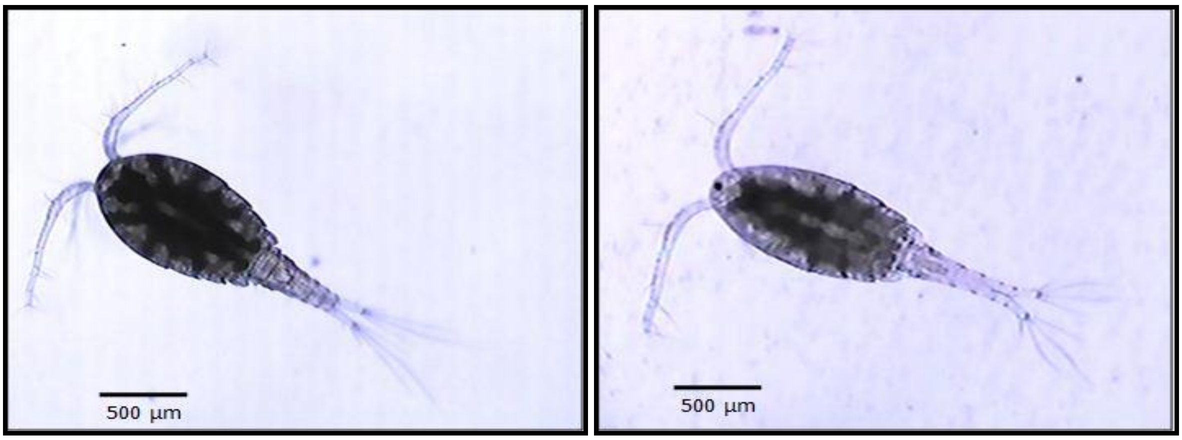
2.3 Statistical analysis

Tukey test was applied to determine the difference in predation of cyclopoid species among different types of mosquito larvae and different larval stages and also for comparison of laboratory and simulated trials.

3. Results

3.1 Identification of copepod species and mosquitoes from collected water samples

The zooplankton samples having copepods collected from fish ponds of Ludhiana district were observed under stereomicroscope and binocular microscope and identified up to order level by following the standard keys [9, 10]. The morphological studies of copepods revealed the presence of two orders of copepods in water samples collected from fish ponds of Ludhiana and these orders were calanoida and cyclopoida. Our target was to search the predatory cyclopoids (having body length ≥ 1.00 mm) and in the present study, two types of large sized cyclopoid copepods were found from these water samples which were identified as *Mesocyclops aspericornis* [12] with body size near about 1.08 mm and *Cyclops vicinus* with body size approximately 1.06 mm (Fig. 1). The species level identification was also confirmed by the zooplankton expert (mentioned in materials and methods).



(a) (b)

Fig 1; Identified large sized cyclopoid copepod species
(a) *Mesocyclops aspericornis* (body size approx. 1.08 mm)
(b) *Cyclops vicinus* (body size approx. 1.0 mm)

Identified cyclopoid species i.e *C. vicinus* and *M. aspericornis* were maintained separately for their pure culture and used for testing of their predatory potential against 1st, 2nd and 3rd instar larvae of *Culex, Anopheles* and *Aedes* collected from the water samples.

3.2 Predatory potential of *M. aspericornis* and *C. vicinus* against different types of mosquito larvae under laboratory conditions

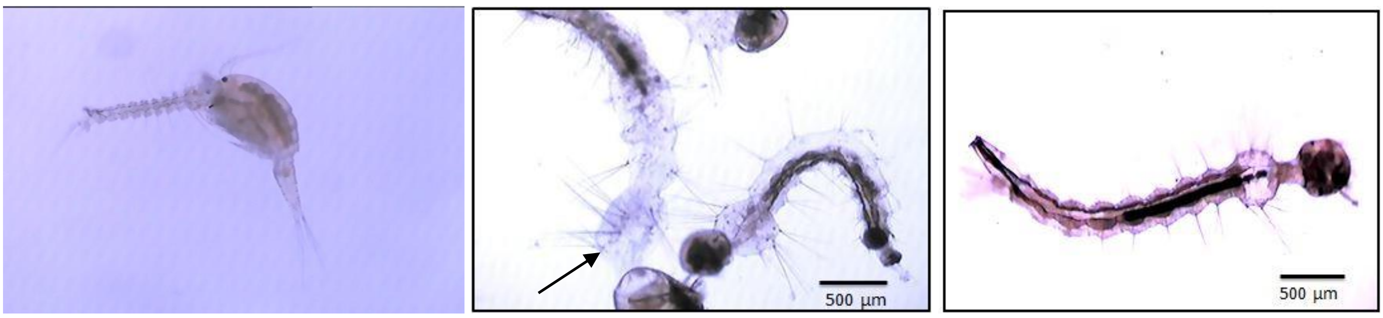
When identified two cyclopoid species were tested for their predatory potential against various larval stages of *Culex, Anopheles* and *Aedes.* No larval killing (even 1^{st} instar of any of the tested larval type) was observed with *C. vicinus* during the present research. However, *M. aspericornis* was capable to kill mosquito larvae showing preferred predation of specific type of larvae and even at specific stage/instar (Table 1). Single *M. aspericornis* was found to kill on an average 9.07±1.93 (ranging from 0-19) 1^{st} instar *Culex* larvae, 0.07±0.04 (ranging from 0-1) 1^{st} instar *Anopheles* larvae and 24.27±3.95 (ranging from 3-50) 1^{st} instar *Aedes* larvae within 24 hours. This predatory potential of *M. aspericornis* towards *Aedes* larvae was found to be statistically higher (p<0.01) in comparison with other two types of larvae (Table 1), indicating the preference of this cyclopoid species for *Aedes* larvae. Thus, average per cent mortality of *Aedes* 1^{st} instar larvae due to predation by *M. aspericornis* was calculated out to be statistically high i.e 49.47±7.86 (p<0.01) followed by *Culex* larvae (18.13±3.85) and least killing of *Anopheles* 1^{st} instar larvae (0.13.13±0.09). Further, the rate of killing of 1^{st} instar *Aedes* larvae by single *M. aspericornis* within 24 hours was found be statistically higher (p<0.05) i.e. 24.27±3.95 as compared to that of 2^{nd} instar (14.19±6.25). However, no predation of 3^{rd} instar larvae of *Aedes* was reported by *M. aspericornis*. In case of 2^{nd} and 3^{rd} larval stges of *Culex* and *Anopheles*, no larval killing by *M. aspericornis* was observed (Table 1).

Table 1: Predatory potential of single *Mesocyclops aspericornis* against different larval instars of *Culex*, *Anopheles* and *Aedes* under laboratory conditions

Parameters	Type of larvae		
	Culex	***Anopheles***	***Aedes***
1^{st} instar larvae (n=50)			
Number of larvae killed/ cyclopoid/24 hours	9.07±1.93 ^{b}	0.07±0.04 ^{c}	24.27±3.95 ^{as}
Range of larval mortality	0-19	0-1	3-50
Percent larval mortality	18.13±3.85 ^{b}	0.13±0.09 ^{c}	49.47±7.86 ^{as}
2^{nd} instar larvae (n=50)			
Number of larvae killed/ cyclopoid/24 hours	0.00	0.00	14.19±6.25 ^{a}
Range of larval mortality	0.00	0.00	0-20
Larval mortality (%)	0.00	0.00	23.38±12.50 ^{a}
3^{rd} instar larvae (n=50)			
Larval mortality	0.00	0.00	0.00

- n represents number of larvae taken
- Each experimental trial was run 10 times and given values are Mean ± S.E.
- Figures followed with superscripts "a, b and c' indicate significantly different (p<0.01) killing of different types of mosquito larvae by *M. aspericornis*
- Superscript 's' indicate significantly higher value (p<0.01) of killing of 1^{st} instar *Aedes* larvae in comparison to that of its 2^{nd} and 3^{rd} instars by *M. aspericornis*

During the present study it was observed that starved *M. aspericornis* attacked the first instar *Aedes* larvae within few seconds and mainly captured the larvae from thorax region. They pierced and cut the larvae into pieces by using their strong mandibles (Fig 2).



(a) (b) (c)

Fig 2: Damage caused by *Mesocyclops aspericornis* to 1^{st} instar *Aedes* larvae
(a) *Aedes* larvae captured by *M. aspericornis*
(b) Damaged larval head (→)
(c) Control/Normal *Aedes* larva

3.3 Predatory potential of *M. aspericornis* against 1^{st} instar *Aedes* larvae under simulated conditions

As *M. aspericornis* showed efficient predatory potential against 1^{st} instar *Aedes* larvae in laboratory, therefore, further trials under simulated conditions were conducted on *Aedes* larvae only. To perform this trial three types of containers were used viz. plastic cups, earthen pots and rubber tyres. When *M. aspericornis* and *Aedes* 1^{st} instar larvae were kept in plastic cups under simulated conditions, single cyclopoid killed on an average 9.34±6.77 *Aedes* larvae out of 50 after 24 hours and 1.84±1.02 within next 24 hours. Thus, on an average total number of larvae killed in plastic cups after 48 hours was 11.17±5.78 larvae/cyclopoid, resulting in average per cent mortality of 20.67±12.20. Similar predation trend was observed by *M. aspericornis* when larvae were kept in other two types of containers i.e. earthen pots and rubber tyres and the difference in per cent larval mortality was found to be statistically non-significant among the three types of containers kept under simulated conditions (Table 2).

Table 2: Predatory potential of *Mesocyclops aspericornis* against 1st instar *Aedes aegypti* larvae kept in different types of containers under simulated conditions

Type of container	Average number of larvae killed /copepod (n=50)			Larval mortality after 48 hours (%)
	Within first 24 hrs	Within next 24 hrs	Within 48 hrs	
Plastic cups	9.34±6.77	1.84±1.02	11.17±5.78	20.67±12.20 [a]
Earthen pots	7.33±3.41	4.50±2.84	11.83±1.36	23.67±2.72 [a]
Rubber tyres	7.34±1.46	1.84±0.44	9.17±1.88	18.33±3.76 [a]

- n represents number of larvae taken
- Each experimental trial was run in triplicate and given values are Mean ± S.E.
- Figures followed with superscript 'a' represents non-significant difference (p<0.05) in killing of *Aedes* larvae by *M. aspericornis* kept in different types of containers

4. Discussion

Copepods are tiny crustaceans, which thrive abundantly in most aquatic habitats like lakes, oceans, temporary ponds, puddles, tree holes etc. In nature only 10% of places with water, where mosquito breeds might contain natural population of copepods, which can drastically reduce the survival of mosquito larvae [13]. There are over 13,000 species of copepods, currently categorized in 8 major groups or orders and three orders which dominate in fresh waters are Calanoida, Harpacticoida and Cyclopoida. The calanoids are mainly herbivorous, the harpacticoids are omnivorous and most of the cyclopoids are predators with the large sized ones preying on 1st instar and sometimes 2nd instar mosquito larvae [14]. Our earlier survey at various standing water bodies has also documented the co-existence of mosquito larvae and copepods in different types of standing water bodies and the predominance of cyclopoid copepods in fish ponds of Punjab, India [15].

Most species of cyclopoids are too small (0.3-1.2 mm body length) to prey on even the smallest/1st instar mosquito larval stage, but other large species of cyclopoids (having body length >1.0 mm) attack and consume newly hatched mosquito larvae without hesitation [6]. Therefore, in the present study the large sized cyclopoids were identified from the water samples collected from fish ponds. Two species of large sized cyclopoids i.e. *M. aspericornis* and *C. vicinus* were found and cultured in the laboratory under controlled conditions. Out of these two types of cyclopoids, *C. vicinus* showed no predation of any type of mosquito larvae indicating its incapability to be used as predator of mosquito larval population. However, the other cyclopoid species *M. aspericornis* showed predatory preference among different types of mosquito larvae like efficient killing of *Aedes* 1st instar larvae (24.27±3.95 larvae/*Mesocyclops*/24 hours) was seen by this cyclopoid species as compared to *Culex* (9.07±1.93 larvae/*Mesocyclops*/24 hours) and *Anopheles* larvae (0.07±0.04 larvae/*Mesocyclops*/24 hours) as shown in table 1. There are certain convinicing facts about the efficient predation of *Aedes* larvae by *M. aspericornis* like: 1) *Aedes* larvae are bottom feeders thus preferrred by the *Mesocyclops* which are benthic in nature 2) they are more active and show whip like and circular movements making them more prone to be captured by predators 3) body segments of *Aedes* larvae are broader which might help in their easy capturing [16]. On the other hand, *Culex* and *Anopheles* larvae faced little predation because of their column/surface feeding behavior, also the presence of bristles and thick cuticle of *Culex* and presence of palmate hair on surface of *Anopheles* larvae might help them in escaping from the predation by *Mesocyclops*, thus reducing its capturing ability [6]. This fact regarding the mosquito specific predation by cyclopoids has also been observed by various researchers [5, 6, 8].

Not only the type of mosquito but the stage of larvae also plays an important role in making the cyclopoid species a potent predator as observed in the present study. *M. aspericornis* efficiently killed 1st instar *Aedes* larvae as compared to 2nd instar and no killing of 3rd instar, this is apparently due to the large size of prey and active movements of the older larval instars of mosquitoes [17]. The predatory cyclopoids show distinct prey selectivity behavioral patterns and these patterns are influenced by many attributes of the prey such as morphology, behavior and taste [18, 19]. Out of total 71 recognized species of this genus *Mesocyclops*, 17 species have been assessed showing the best potential for mosquito control in tropical regions. Out of these 17 species at least nine species are being used as predators of mosquito larvae and the most often used are *M. albidius, M. longisetus* and *M. aspercornis* [8, 20].

It was observed that starved *M. aspericornis* attacked the first instar larvae within a few seconds and mainly captured the larvae near the joint region of head and thorax (Fig. 2a). The larva is cut generally at this region as can be clearly seen under microscope (Fig. 2b). The cyclopoids pierced and crammed the larvae into pieces by using their strong mandibles leading to larval damage. Hunger level is an important determinant of the predatory efficiency of copepods which in turn affects cyclopoid feeding rate, predation behavior and the selection of profitable prey [18]. Therefore, predators respond with behavior that is state-dependent, such as prey density [21, 22], hunger level and gut fullness [23]. Predation behavior affects further foraging, because well-fed predators take a longer time to feed and react less efficiently to encounter prey than when they are hungry [24]. *Mesocyclops* are also wasteful killers with the capacity to kill more mosquito larvae than they actually ingest. If larvae are numerous they eat a small part of each larvae and single cyclopoid has the capacity to kill 30-40 larvae/day, which is far more than they actually eat. This behaviour increases their larval control utility [19].

In simulated trials (performed in plastic cups, earthen pots and rubber tyres) cyclopoid predation rate was found to be higher on 1st day, while less on second day in all the three types of containers (Table 2). This was because 1st instar larvae moulted to 2nd instar stage generally after 24 hours and size of 2nd instar was large as compared to 1st instar. Another observation made was lesser predatory potential of *M. aspericornis* in simulated trials as compared to laboratory trials (Table 1 and 2), this might be due to the presence of alternative food i.e *Paramecium* which was added along with the *Aedes* larvae in these containers. Reduction in predation rate of cyclopoids on mosquito larvae in the presence of abundant alternative food has also been reported [21]. The efficacy of *Mesocyclops* in supressing larval populations of *Ae. aegypti* has been demonstrated in field trials also, like in Louisiana [6], Vietnam [8] and Venezuela [25]. Potent reduction in *Ae. aegypti* population has been observed in the water storage containers having established population of *M. aspericornis* [13]. Also, the New Orleans Mosquito Control

Board (NOMCB) has successfully eliminated *Ae. albopictus* production in thousands of tyres by introducing *M. longisetus*[8]. Thus, biological control, using such kind of natural enemies of *Aedes*, appears to be an alternative approach to the synthetic insecticides being used for mosquito killing. Several species of copepods, particularly those in the genus *Mesocyclops*, have shown promise as biological control agents for container-breeding mosquito i.e *Aedes* spp.

5. Conclusion

In summary, this study indicated the presence of large sized cyclopoid species i.e *M. aspericornis* in fish ponds of Ludhiana district, Punjab (India) and also revealed good predatory potential of this cyclopoid species specifically against *Aedes* 1st instar larvae. So, *M. aspericornis* can be used as bio-control agent against dengue spreading *Aedes* mosquito for its testing at field level in future.

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