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Elimination of Dengue by Control of Aedes Vector Mosquitoes (Diptera: Culicidae) Utilizing Copepods (Copepoda: Cyclopidae)

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Abstract

This paper reports on the information and result of long-term laboratory and field studies on copepods (Copepoda: Cyclopidae) as predators for mosquito control inhabiting in tropic and subtropic environments. Mosquitoes have long been vectors of numerous diseases that affect human health and well-being in many parts of the world. Reducing the use of pesticides against insect vectors is one of the big demands of the society because public has always been against the heavy use of insecticides. Copepods are natural and tiny shrimp-like crustacean with a hearty appetite for feeding on mosquito larvae in water holding areas. The copepods thrive in fresh and marine water, and are valuable tool in battling mosquitoes in artificial containers, roadside ditches, small water pools, clogged downspouts and other wet areas that can breed plenty of mosquitoes. These are especially helpful tools in fighting mosquitoes near public places, where use of certain pesticides is restricted. Copepods are relatively easy to culture, maintain and deliver to the target areas, but getting the cultures started requires some effort and time. Copepods are more efficient predator of younger than of older larvae of mosquito and predation drops considerably for 4 days and older larvae. Copepods though prefer to prey on younger larvae, yet also increasingly attack on older larvae as greater predator densities reduce the supply of younger ones. Recent trials show that each copepod might destroy forty larvae per day, cutting Aedes breeding by 99 to 100 percent and in practice these can best be used where most of the local mosquito problem is due to Aedes breeding locations. Once established, copepods are able to survive and reproduce well to maintain viable populations under a wide variety of field conditions. The use of these biological control agents is only one small part of a statewide integrated approach to mosquito control, and not a replacement for long-established procedures. The future additional research can build up new ways of producing and keeping alive copepods that might target specifically dengue vector mosquitoes, but few realistic problems still need to overcome.

Keywords

Dengue, Copepods, Cyclopoida, Mosquito Control, Biological Control

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1. Introduction

Throughout the world, mosquitoes transmitted diseases such as dengue, malaria and yellow fever are serious threat to human health. Moreover, mosquitoes are also severe annoyance with noteworthy interference on the economy of state and quality of public life. Biological control of mosquito is a significant opportunity for the future control of vectors borne diseases, principally in view of existing limits on use of pesticides, continuing problems of resistance to chemicals and management of habitat. The public is normally not always aware of some of the things protecting them, particularly a variety of biological control agents making

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attractive to the countries. Quite a lot of species of copepods (Copepoda: Cyclopidae), in various genera have shown assurance as biological control agents for control of mosquitoes (Sarwar, 2014 a; 2014 b; 2014 c; 2014 d).

The Cyclops is one of the most common genera of freshwater copepods, belongs to Phylum Arthropoda, Subphylum Crustacea, Subclass Copepoda, Order Cyclopoida, Family Cyclopidae, and comprising over 400 species. Together with other similar-sized non-copepod fresh-water crustaceans, especially Cladocera, these are commonly called water fleas having a single large eye, which may be either red or black in cyclops. Cyclops individuals may range from 0.5- 5 mm long and are clearly divided into two sections. The broadly oval front section comprises the head and the first five thoracic segments. The hind part is considerably slimmer and is made up of the sixth thoracic segments and the four legless pleonic segments. Two caudal appendages project from the rear side of body and have 5 pairs of legs. Owing to less than a couple millimeters length, these are barely visible to the naked eye, shaped like a teardrop, have long antennae and an exoskeleton that is so thin that it is largely transparent. The long first antennae, 2 in number, are used by the males for gripping the females during mating. Afterwards, the female carries the eggs in two small sacs on its body. The larvae or nauplii, are free-swimming and unsegmented. Cyclops has a cosmopolitan distribution in fresh water, but is less frequent in brackish water. It lives along the plant-covered banks of stagnant and slow-flowing bodies of water, where it feeds on small fragments of plant material, animals or carrion. It swims with characteristic jerky movements. Cyclops has the capacity to survive unsuitable conditions by forming a cloak of slime and average lifespan is about 3 months (Marten, 1986).

Tiny copepods are aquatic crustaceans that are widespread in both fresh and salt water habitats. The kinds of copepods proposed for mosquito control are referred to collectively as cyclopoid copepods. These are voracious predators of mosquito larvae and as such can be used to control mosquito production from water holding areas. Particularly, these are natural enemies of the first and second instars (the smallest sizes) of mosquito larvae. The copepods attack and eat larvae, but can also survive on other food sources in water, so they can live for long periods even after these have eaten all the larvae, waiting for more progeny to hatch. Several species of copepods, particularly those in the genus Mesocyclops, have shown promise as biological control agents for container-breeding mosquitoes. A notable success in the use of copepods for reduction of disease vectors is that which reports effective control of Aedes vectors of dengue with copepods of the genus Mesocyclops (Kay et al., 2002). Two kinds, *Mesocyclops longisetus* and *Macrocyclops albidus,* are the ones that behave most aggressively, destroying larvae that come near even when these do not eat them. Recent tests with the copepod *M. albidus* in the laboratory and in field conditions showed similar results, with almost a 90 percent reduction in larvae. Tests have been done by adding them to water in containers, and so far, these have been fairly successful. Recent trials showed that these kill mostly first instar larvae of Aedes mosquitoes, but each copepod might destroy forty larvae per day, cutting down Aedes breeding by 99 to 100 percent. These are less effective with Anopheles and least effective with Culex larvae. In practice, these would best be used where most of the local mosquito problem is due to Aedes breeding in known locations, such as pools or water storage containers (Marten et al., 2000; Byer, 2004).

2. Biological Control of Mosquito Larvae Using Copepods

Past studies on use of Cyclopoida have reported varying results depending upon the copepods, mosquito species, weather, and the physical and biological milieu of the water holding containers and areas. Six species of cyclopid copepods have been tested for biological control of *Aedes albopictus* larvae in discarded tires. Six to 8 weeks after introduction, *Diacyclops navus, Acanthocyclops vernalls, Mesocyclops ruttneri* and *Mesocyclops edax* reduced the number of *A. albopictus* larvae by 83, 90, 95 and.96%, respectively. *Macrocyclops albidus* and *Mesocyclops longisetus* are the most effective species. Six to 8 weeks after introduction, *M. albidus* reduced *A. albopictus* larvae by 99%. Three months after introduction, *M. albidus* reduced *A. albopictus* larvae by 100%, and *Mesocyclops longisetus* reduced *A. albopictus* by 99.8%. *Macrocyclops albidus* and *M. longisetus* are equally effective at eliminating *Aedes aegypti* (L.), and *A. triseriatus* larvae (Marten, 1990 a). There is also evidence that natural populations of cyclopoids eliminated Aedes and Anopheles larvae from ground water habitats (Marten et al., 1989; Marten, 1989; 2000). Further filed trials have been conducted to determine whether copepods can usefully destroy larval Stegomyia mosquitoes. Copepods (*Macrocyclops albidus*) have been released into each of about 200 discarded tires arranged in 2 stacks of about 100 tires, while a third stack remained untreated. Larval *A. albopictus* that have been numerous in the treated tires at the beginning of the experiment virtually disappeared within 2 months. Adults disappeared about 1 month later and remained scarce for at least another year. These predators, however, do not reduce the abundance of *Culex salinarius*, which is another type of mosquito (Marten, 1990 b).

In laboratory tests, four different strains of *Mesocyclops* *aspericornis* (Daday) showed potential as biological control agents of *A. aegypti* mosquito larvae, but have not been as effective against Anopheles or Culex. In *c*ontrast, the larger *Mesocyclops longisetus* (Thiebaud), killed 100% of *A. aegypti* and *Anopheles farauti* (Laveran) at larval densities of 200 per liter and *Culex quinquefasciatus* (Say) at 25 per liter. In cage simulations with *A. aegypti* and Mesocyclops*,* both copepod species eliminated all immatures in earthenware pots by week 3. Owing to the lack of replacement, all *A. aegypti* adults subsequently died by week 8 or 9. Although both *M. longisetus* and *M. aspericornis* showed maximum reproductive potential at 25°C, yet breeding occurred from 20 to 35°C (Kay et al., 1992). A field trial used copepods in water tanks, tires and vases to control *A. aegypti.* It has been found that the most effective control is in the cemetery vases, with 67.5% reduction over the 3 month study period (Gorrochotegui-Escalante et al., 1998). Copepods have also been used to successfully control *A. aegypti* populations in Argentina (Marti et al., 2004) and *A. albopictus* population in Japan has been effectively controlled by Macrocyclops copepods (Dieng et al., 2002).

The cyclopoid copepod *Macrocyclops albidus* (Jurine) has been tested as a potential biological control agent of mosquitoes in laboratory microcosms, in controlled field conditions, and in a field experiment using discarded tires. The predator is highly efficient in controlling mosquitoes in all three settings, reaching close to 90% reduction in larval survival under field conditions and exceeding the recommended predation rates for effective mosquito control in laboratory experiments (effective on 1-4 day old larvae). Alternate food and habitat structure significantly influenced the predation rates on mosquito larvae. Once established, the copepod is able to maintain reproducing populations in the field for the duration of the experiments (Jorge et al., 2004). The cyclopoid crustacean *M. longisetus* has been evaluated for its predatory potential to reduce container-inhabiting mosquitoes in backyards. Mosquitos *A. albopictus*, *A. triseriatus* and *Culex quinquefasciatus* have been the predominant species collected from containers. At an initial inoculation rate of 120 copepods per container, *M. longisetus* populations eliminated resident mosquito larvae for a minimum of 14 wks in 30 liter plastic buckets, up to 29 wks in 0.4 liter ceramic flowerpots and 0.3 liter glass jars depending on species. Copepod populations generally peaked 13 wks after introduction (August) in ceramic flowerpots and glass jars, and about 1 month later in tires, plastic buckets and plastic flowerpots. At the time of peak abundance, average predator numbers ranged between 900 (glass jar) to > 3000 (30 liter bucket) individuals per container. Although all mosquito species have been eliminated from all containers, sometime during the 35 wks study, yet *M. longisetus* appeared to preferably prey on Aedes larvae compared with Culex. Operationally, the use of *M. longisetus* as a tool for control of container-inhabiting mosquitoes in urban or suburban settings proved to be relatively inexpensive, required little labor for colony maintenance, is easily transported and smoothly applied to the target areas (Soumare and Cilek, 2011).

As the most successful type of invertebrate used for mosquito larva control is the cyclopoid copepods, so most notably Mesocyclops that are one of the most numerous multicellular organisms on earth that can be found in many geographical locations, and therefore the use of copepods for mosquito control does not require exotic introductions. Because of their small size, copepods mainly kill the first instar larvae, and these prefer Aedes larvae over Anopheles and Culex larvae. Copepods can live for 1-2 months, are quite hardy and they self-replicate readily. Because these eat a variety of aquatic prey, they can maintain populations in water storage containers even if mosquitoes are not found (Marten and Reid, 2007; DeRoa et al., 2002). These can also be easily moved from one container to other container habitats; therefore, they offer the potential of sustainable mosquito control. Furthermore, copepods can be easily and cheaply mass produced and transported, even under field conditions where they are required. Nam et al., (2000) used a method using plastic garbage bins in which thousands of copepods could be produced in just 3 weeks. They then transported these copepods to the various field locations using hollowed polystyrene blocks.

The major success story for the use of copepods against dengue vectors comes from a study carried out using Mesocyclops. As the primary control measure, copepod has been able to reduce *A. aegypti* levels to 0-0.3% of baseline estimates and *A. albopictus* to 0-14.1% of baseline levels (Kay et al., 2002). The authors report that Aedes mosquitoes have been eliminated from several study communes following country-wide control program, and no dengue has been detectable in the three treated areas, but dengue transmission is still evident in the control areas (Nam et al., 2005). In his way, dengue has not been reported in the treated areas for years but dengue transmission remained in the untreated areas (Kay and Nam, 2005). Use of copepod Mesocyclops is also proving to be sustainable, 7 years after official involvement has been ceased. Copepod Mesocyclops are still being used by community members to keep Aedes populations at bay, and local transmission of dengue has been eliminated in areas where these are being used (Kay et al., 2010).

Predation efficiency of copepods has been reported to drop off considerably with older mosquito larvae, the effects of habitat structure and alternative prey for predation (Tietze et al., 1994). One of the characteristics that make some cyclopoid copepods species good candidates for biological control of mosquitoes is their broad prey base. This may allow some of these copepod species to survive in containers even when mosquito larvae are absent and may facilitate population establishment in new containers (Jennings et al., 1994). Thus, copepods are the group with the most potential, and these are very cheap to yield as biolarvicides because today these show a great potential to be used in the Integrated Vector Management (IVM) control programs against *A. aegypti* worldwide. When these organisms are placed in container habitats, decorative ponds and pools, these prey on mosquito larvae, and effectively prevent mosquito development. The addition of copepods into large water-storage tanks has been successful in limiting dengue transmission. Small copepod crustaceans of the genus Mesocyclops can be removed from a pond or lake and placed in water containers to kill Aedes mosquito larvae. The technique is simple, cheap and can be very effective. A single copepod has been observed to kill 15 to 20 first and second instars larvae in a single day. A single introduction can produce effective larval control from several weeks to several years, depending on the larval habitat.

3. Public Health Importance of Copepods

There are some slight risks with introducing of copepods on a large scale, to which scientists are still evaluating and they should focus on their public health issues. Cyclops is intermediate host of dracunculiasis (guinea-worm disease- this is a helminth human parasite that infects peoples when they ingest infected Mesocyclops in drinking water) and fish tapeworm (*Diphyllobothrium latum*) infection. Copepods themselves are also associated with the spread of some of human diseases such as cholera, but these cannot spread it unless the disease is already present in the area. Another disadvantage is that these copepods are sensitive to chlorine in the water (Marten and Reid, 2007). Nevertheless, despite these disadvantages Mesocyclops has been sustainably used to almost eradicate dengue vector mosquitoes (Kay et al., 2010).

Under certain circumstances when copepods become public health problems, these can be controlled using physical (straining of water through piece of fine cloth is sufficient to remove cyclops and can also be killed easily by boiling at 60°C), chemical (chlorine destroys guinea worm larvae and Cyclops in strength of 5 ppm, though this concentration gives bad odor and taste to water, excess chlorine can be removed by dechlorination, lime at dosage of 4 gm per gallon of water can be used, Temefos kills cyclops at concentration of 1 mg per litter), biological (small fishes like Barbell and Gambusia feed on cyclops to eradicate dracunculiasis) and engineering (provision of drinking water through piping water supply, use of tubewells and abolition of stepwells) methods that are effective measures on community level (Marten, 1986).

4. Rearing of Copepods for Mosquito Control

Copepod cultures are relatively easy to maintain as can be seen below, but getting the cultures started requires some effort and time, however, once established, copepods are able to survive and reproduce well to maintain viable populations (Jorge and Sheila, 2004; Suarez et al., 1992).

4.1. Field Collection of Copepods

Copepods for culture start-up can be collected from ponds, ditches and other standing water sources. These can be collected by quickly dipping a suitable container (mosquito dipper) in the water, particularly near submerged vegetation. After dipping, slowly pour out 2/3 of the water in the container, and look at the remaining water for signs of the copepods, which are small, can be seen swimming with jerky movements in the collection vessel, and then quickly pour the copepods present, into a clean container. Copepods can also be collected by passing water through two in-line sieves, either by pumping or by scooping water with a bucket and pouring through the strainers. The top sieve can be an ordinary kitchen strainer to capture large debris and copepods can be captured in the second sieve of 200 µm mesh netting covering a second strainer and secured by the edges to the strainer with a rubber band. After filtering through this set-up, either remove the netting and invert in a container of clean water, or turn the second sieve set-up upside down over a clean container and rinse with clean water.

4.2. Start-Up of Copepod Cultures Inside

Gravid females copepod can be easily recognized because these carry their eggs externally on both sides of their bodies. To start culture, set up several small containers (disposable food containers) about 3/4 full of water and introduce a single gravid female in each by picking up the female with a pipette and placing it in the culture container. Copepods can be reared in pools (plastic pools), garbage cans or other suitable containers. Fill the pools with water, introduce several dozen adults and monitor these containers to determine if the copepod's population is growing after 2-3 weeks. Add approximately 1000 ml of Paramecium culture and 100 grains of wheat into each of the rearing pools. The copepods will readily predate on the Paramecia, and the Paramecia feed on the wheat grain. Place the pools to receive light (natural or artificial) for at least a part of the day, but keep away from direct sun. As the wheat seed decomposes, some will start floating to the surface of the water and should be removed. Pools should be emptied and cleaned at least once yearly typically in late spring to avoid extreme temperature creating the chance of mortality.

4.3. Paramecium Cultures

Plastic milk cartons (1 gallon or 1/2 gallon) work well for culturing of Paramecium, as well as 5 gallon water jugs are also appropriate. Add 25 ml of the *Paramecium caudatum* stock culture to 1 gallon of spring water, then add approximately 12 grains of wheat seed per gallon of culture and a small pinch of yeast, and wait for about two weeks. Save about 500 ml water of each jug, refill with water and add wheat seed and yeast as before to start a new culture.

4.4. Rearing of Copepods Outside

When copepods are cultured outside, the culture needs to be covered at least 3 ft, off of the ground level. Aquaria or similar vessels are not ideal for copepod rearing outside. It is a good idea to set up several pools and to maintain additional cultures in smaller containers at a separate location so that new cultures can be started in case of loss of original cultures.

4.5. Identification and Predation Efficiency Testing of Copepods

Ideally, copepods captured in the field should be identified to species level and it requires an expert taxonomist for reliable identification. Before a heavy time investment is made to rear copepods for mosquito control, their reliable identification is outmost as all copepods species are not effective control agents. For testing predation rates, obtain first instar mosquito larvae, place 500 newly hatched larvae in a 200-500 ml container half full with water and add 8-10 adult copepods. Let it sit for 24 hrs, and then count how many mosquito larvae are left. For effective mosquito control, a single copepod should be able to consume approximately 30 or more first instar larvae per day.

5. Conclusion

Biological approaches are considered as alternatives to chemical control of mosquito populations. For example, predatory crustaceans called copepods are promising candidates for control of mosquito larvae because these are widespread and highly effective predators that are capable of establishing and maintaining their populations under a wide variety of field circumstances. Copepods are able to manage mosquito populations using an integrated control approach, stressing environmental safety, economics, efficacy, research and surveillance in order to protect the health and welfare of county residents and the many tourists who visit abroad during the months when mosquitoes are active. Once the populations of copepod are building up in a container, these are able to maintain viable reproducing inhabitants for successful and excellent biological control of mosquitoes. Copepods are very useful in biological control of *A. aegypti* and *A. albopictus,* but also successful to manage the populations of Culex and Anopheles. These predators are most effective to predate on 1 to 4 days old mosquito larvae, but alternate food and habitat structure significantly influence the predation rates on prey. However, this characteristic does not appear to negate its effectiveness as a mosquito larval predator. The technology for culturing and field application of copepods is appropriate, its costs appear to be modest and little environmental risk is evident. The states should offer assistance to county members for mosquito control programs and help to reduce their dependence on insecticides, by providing them a variety of natural tools to deal with mosquito issues. Further tests are needed to determine the effectiveness of these predators in larger bodies of water such as ditches and swales, as available data on such water holdings are scanty in tropics and subtropics.

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