Scanning electron microscopy of damage caused by *Mesocyclops thermocyclopoides* (Copepoda: Cyclopoidea) on larvae of the Dengue fever vector *Aedes aegypti* (Diptera: Culicidae)

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Abstract: Dengue fever is a mosquito-borne viral disease, whose main biological vector is *Aedes aegypti*. This mosquito colonizes tropical areas where the disease is endemic. The most obvious action against dengue is attacking its vector. Biological control appears to be an alternative approach, using natural enemies of the mosquitoes, such as predatory copepods. Thus, the morphological study of the damage caused by copepods is important to understand its predatory capacity. Twenty-five *A. aegypti* larvae were exposed to the copepod *Mesocyclops thermocyclopoides* and the damage caused by the copepods was evaluated using scanning electron microscopy. The larvae showed damage mainly at the anal segment, the siphon and the abdomen; only three attacks to the head were observed. The size of the siphon might be of importance in determining whether or not a copepod will attack a mosquito larva. Rev. Biol. Trop. 54 (3): 843-846. Epub 2006 Sept. 29.

Key words: *Mesocyclops thermocyclopoides, Aedes aegypti,* predation, larval damage.

Dengue and dengue hemorrhagic fever are mosquito-borne viral diseases, which are endemic in more than 100 countries in the tropical and sub-tropical territories of the world, causing an estimated 100 million cases annually (Gubler 1998, Keating 2001). Since there are neither antiviral drugs available to eliminate this virus nor an effective vaccine, the logical approach against these diseases is controlling the mosquito vector (Gubler 1998). During the 1950s, chemicals such as DDT were the main weapons used in this battle (Breakley *et al*. 1984), and whose apparent success was evident 30 years ago with the eradication of *Aedes aegypti* from 18 countries of the American continent (Kuno 1995, Guzman and Kouri 2002). At the present time, the mosquito has once again colonized these territories and dengue has re-emerged as an endemic disease. There are many causes that explain the re-emergence of dengue, such as the natural selection of strains resistant to insecticides, as well as global changes, such as an increase in international flight, the migration of people from rural to urban areas and uncontrolled re-urbanization (Guzman and Kouri 2002). The peak of the plastics industry around the world could be another factor associated with the re-emergence of dengue, because of the huge quantity of discarded plastic containers that provide an infinite number of artificial breeding grounds for the mosquitoes.

Biological control, using the natural enemies of *Aedes*, appears to be an alternative approach to the systematic failure of use of insecticides (Lardeux *et al*. 2002). There is a vast array of agents used in the biological control of mosquitoes, including copepods. These agents are microcrustacea, present in fresh water worldwide. *Mesocyclops* is one of the genus of copepods that has most been studied as an antagonist of mosquito larvae and whose effectiveness has been demonstrated in different countries, including the United States (Marten 1990), Honduras (Marten *et al.* 1994), Vietnam (Nam *et al.* 1998), and the French Polynesia (Lardeux *et al.* 2002).

Mesocyclops thermocyclopoides is a very common species in Costa Rica (Collado *et al*. 1984, Hernández-Chavarría and Schaper 2000) and was evaluated as a biological control agent against *Aedes*. This copepod feeds on the 1st and 2nd instars of the mosquito larvae, fatally wounding about seven individuals per day (Shaper and Hernández 1998). Although there has been considerable work done on the effects of this and other species of copepods on the populations of *A. aegypti* and *A. albopictus* (Brown *et al*. 1991, 1996, Marten *et al*. 1994, Tietze *et al*. 1994, Schaper and Hernández 1998, Dieng *et al*. 2002), more detailed studies of the effects on the individual seem to be necessary. So far, little is known about the morphological damage caused by the copepod. It is assumed that an evaluation of the injury caused by the copepod might help to explain why some species of mosquitoes are attacked (such as *Anopheles* and *Aedes* spp.) and others, such as *Culex* spp., are not.

Over the span of three days, five *A. aegypti* larvae at the 1st instar stage were placed in plastic capsules with 0.5 ml of water and one adult female *M. thermocyclopoides*. After 5 h of incubation at room temperature, the organisms inside the capsule were fixed with 0.1 ml of 25 % gluteraldehyde for at least 2 h at 4 °C. Then the samples were washed in 0.1 mM phosphate buffer three times and post fixed in 1 % OsO_4 for 1 h, washed again and dehydrated in ethanol (30 to 100 %), dried in a critical point dryer, mounted on aluminum studs, covered with gold and analyzed under a Hitachi S-570 scanning electron microscope.

Adult copepods (*M. thermocyclopoides*) and the 1st instars of *A. Aegypti* are almost the same size, as is shown in Fig. 1. The majority (76 %) of the larvae attacked showed damage on the anal segment, the siphon and the last abdominal segments (19 of 25 cases) as is shown in Fig. 1 and 2. In two cases (8 %), the thorax was also eaten, so that only the head capsule remained (Fig. 1). In three (12 %) cases an attack to the head was observed (Fig. 3). Three (12 %) individuals showed lateral bites on the thorax and abdomen. In the 1st instars larvae, the siphon is not strongly sclerotized as in the later instars. Apart from the smaller size, this might make the 1st instars more susceptible to copepod attacks.

If the copepod bites the larvae at the anal segment and the siphon, the consequences are obviously fatal for *A. aegypti* because the siphon is essential for breathing. Also, the lateral damage detected on the larvae could be the result of primary attacks, as several times the copepod was observed to strike the larva several times to immobilize it before starting to feed on it.

The small size of the siphon of a mosquito larva might be important to predict if a copepod can attack the larva or not. *A. aegypti* is susceptible to copepod predation but long siphoned species like *Culex quinquefasciatus* suffer only insignificant attacks by *M. thermocyclopoides*. *Anopheles albimanus*, which does not have a siphon at all, is also susceptible to copepod predation (S. Schaper, pers. obs.).

As Nam *et al*. (1998) demonstrated in Vietnam, a combination of both biological

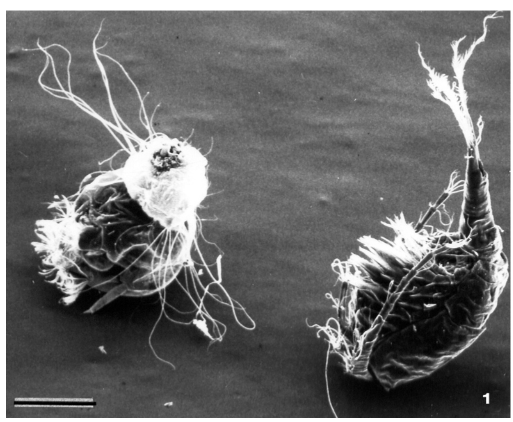

Fig. 1. Comparison of an *Aedes aegypti* attacked larva with the copepod *Mesocyclops thermocyclopoides* (Bar = 0.2 mm).

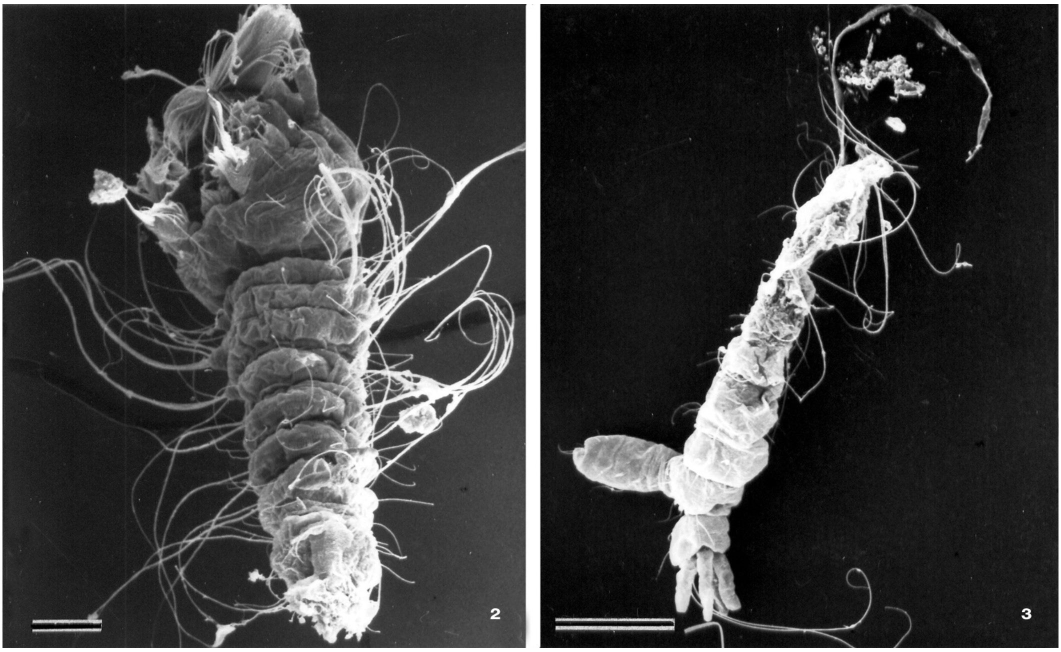

Fig. 2 and 3. Fig. 2. Attacked larva with damage to the abdominal segments, loss of the anal segment and siphon (Bar = 0.1 mm). Fig. 3. Attack to the head (Bar = 0.1 mm).

control using copepods and active community participation proved the most effective intervention to eradicate *Aedes* from an infested territory. An environmental education campaign raised awareness, while recycling programs were established to recycle materials such as used tires, glass bottles, aluminum cans and some plastic recipients. In the experience of Nam *et al.* (1998), copepods were used for biological control, but any isolated intervention would have been effective to eradicate this vector. Nonetheless, it has been determined that an integrated use of all strategies is best in the battle against dengue. A low cost and highly efficient strategy to control *Aedes* and Dengue should include four elements: 1) A combined vertical and horizontal approach that depends on community understanding, 2) Prioritized control according to the larval productivity of major habitat types, 3) Use of copepods (*Mesocyclops*) as biological control agent, 4) Community activities of health volunteers in schools and for the public. As a result of such a strategy, *Aedes* was eradicated from 32 of 37 communities (population 309 730) where no dengue cases have been detected since 2002 (F.H.-C., unp.data).

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RESUMEN

El dengue es una enfermedad viral transmitida por mosquitos, cuyo principal vector es *Aedes aegypti.* Este mosquito coloniza muchas áreas tropicales donde la enfermedad es endémica. La acción más obvia contra el dengue es el ataque a su vector. El control biológico parece una buena alternativa, empleando enemigos naturales de los mosquitos, como los copépodos. Por lo tanto, es importante el estudio morfológico del daño causado por los copépodos para comprender su capacidad depredadora. Veinticinco larvas de *A. aegypti* fueron expuestas a la actividad depredadora del copépodo *Mesocyclops thermocyclopoides*. Mediante microscopia electrónica de rastreo se evaluó el daño causado por los copépodos. Éstos atacaron principalmente el segmento anal, el sifón y el abdomen de las larvas; sólo vimos tres ataques a la cabeza. El tamaño del sifón podría ser de importancia para predecir si los copépodos pudiesen atacar larvas de determinado mosquito.

Palabras clave: *Mesocyclops thermocyclopoides, Aedes aegypti,* depredación, daño larval.

REFERENCES

Breakley, C.J., P.L. Crampton, F.E. Ricket & P.R. Chadwick. 1984. Resistance mechanisms to DDT and Transpermethrin in *Aedes aegypti*. Pestic. Sci. 15: 121-133.

Brown, M.D., B.H. Kay & J.K. Hendrikz. 1991. Evaluation of Australian *Mesocyclops* for mosquito control. J. Med. Entomol. 28: 618-623.

Brown, M.D., J.H. Hendrikz, J.G. Greenwood & B.H. Kay. 1996. Evaluation of *Mesocyclops aspericornis* and *Toxorhynchites speciosus* as integrated Predators of mosquitoes in tire habitats in Queensland. J. Am. Mosq. Control Assoc. 12: 414-420.

Collado, C.D., D. Defaye, B.H. Dussart & C.H. Fernando. 1984. The freshwater Copepoda of Costa Rica with notes on some species. Hidrobiologia 119: 89-99.

Dieng, H., M. Boots, N. Tuno, Y. Tsuda & M. Takagi. 2002. A laboratory and field evaluation of *Macrocyclops distinctus, Megacyclops viridis* and *Mesocyclops pehpeiensis* as a control agents of the dengue vector *Aedes albopictus* in peridomestic area in Nagasaki, Japan. Med. Vet. Entomol. 16: 285-291.

Gubler, D.J. 1998. Dengue and dengue hemorrhagic fever. Clin. Microbiol. Rev. 11: 480-496.

Guzmán, M.G. & G. Kouri. 2002. Dengue: an update. Lancet Infect. Dis. 2: 33-42.

Hernández-Chavarría, F. & S. Schaper. 2000. *Mesocyclops thermocyclopoides* (Copepoda: Cyclopoidea): A scanning electron microscopy study. Rev. Latinoamer. Microbiol. 42: 53-56.

Keating, J. 2001. An investigation into the cyclical incidence of dengue fever. Soc. Sci. Med. 53: 1587-1597.

Kuno, G. 1995. Review of the factors modulating dengue transmission. Epidemiol. Rev. 17: 321-335.

Lardeux, F., F. Riviere, Y. Séchan & S. Loncke. 2002. Control of the *Aedes* vector of the dengue viruses and *Wuchereria bancrofti*: The French Polynesian experience. Ann. Trop. Med. Parasitol. 96: S105-S116.

Marten, G.G. 1990. Elimination of *Aedes albopictus* from tire piles by introducing *Macrocyclops albidus* (Copepoda, Cyclopidae). J. Amer. Mosq. Control Assoc. 6: 689-693.

Marten, G.G., G. Borjas, M. Cush, E. Fernández & J.W. Reid. 1994. Control of larval *Aedes aegypti* in peridomestic breeding Containers. J. Med. Entomol. 31: 36-44.

Nam, V.S., N.T. Yen, B.H. Kay, G.G. Marten & J.W. Reid. 1998. Eradication of *Aedes aegypti* from a village in Vietnam, using copepods and community participation. Am. J. Trop. Med. Hyg. 59: 657-660.

Schaper, S. & F. Hernández. 1998. La Lucha contra el Dengue y *Mesocyclops thermocyclopoides*: un posible control biológico para larvas de *Aedes aegypti*. Rev. Cost. Cienc. Med. 19: 119-125.

Tietze, N.S., P.G. Hester, K.R. Shaffer, S.J. Prescott & E.T. Schreiber. 1994. Integrated management of waste tire mosquito utilizing *Mesocyclops longisetus, Bacillus thuringiensis* var. *israelensis* and Methoprene. J. Am. Mosq. Control Assoc. 10: 363-373.