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THE USE OF THE COPEPOD *MESOCYCLOPS LONGISETUS* AS A BIOLOGICAL CONTROL AGENT FOR *AEDES AEGYPTI* IN CALI, COLOMBIA

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ABSTRACT. We present data on the efficacy of *Mesocyclops longisetus* as a biocontrol agent in controlling *Aedes aegypti* larvae in catch basins in Cali, Colombia. Additionally, we determined some of the features that facilitated the establishment of the copepods in catch basins. Between June 1999 and February 2000, 201 catch basins were treated with an average of 500 adult copepods. The copepods had established in 49.2% of all the basins and they maintained *Ae. aegypti* larvae at low densities until the end of the 8-month study. The corrected efficacy percent was 90.4%. The copepods established in basins located in a flat area as opposed to those in steep areas, exposed to sunlight and with 0–10% of floating organic matter. When the catch basins were contaminated with synthetic washing agents, like detergents, the copepods did not survive. The copepod *M. longisetus* could be incorporated as a biological control agent in an integrated *Ae. aegypti* control program.

KEY WORDS Biological control, *Aedes aegypti,* copepods, *Mesocyclops longisetus,* Colombia

INTRODUCTION

Many strategies, such as vector eradication programs, chemical control measures, environmental sanitation with community participation, and biological control agents, have been used to prevent or control dengue outbreaks. An early approach to control yellow fever and dengue fever was to eradicate the dengue vector, *Aedes aegypti* (L.). The eradication programs failed due to financial, political, technical, and administrative problems. As a consequence, dengue vector populations returned to previous or higher densities, and the mosquito dispersed into new areas (Halstead 1984, Nelson 1986, Clark 1995, Gratz and Knudsen 1995, PAHO 1995, Gubler and Clark 1996). Chemical control was also used to eliminate the vector, but the reduction of female mosquitoes was transitory (Leontsini et al. 1993), and the mosquito developed resistance to many insecticides (PAHO 1995). Environmental sanitation with community participation involved the elimination of all artificial breeding sites of the vector. Community-based approaches provide short-term success, but this control strategy has yet to show a significant impact on vector populations in operational levels (Gubler and Clark 1996). Biological control is a safe strategy and does not have some of the negative environmental drawbacks of insecticides (Howarth 1991). The main drawbacks of biological control are that the predator has to colonize the habitats of its prey in urban environments, there is a lag time for its establishment (Rawlins et al. 1997), and repeated applications are needed for effective control (Laird 1981).

Copepods attack and kill 1st-stage mosquito larvae (Suárez et al. 1991) and can suppress larval populations to very low levels (Marten et al. 1994). Some cyclopoid copepods have been observed to control *Ae. aegypti* larvae in domestic and peridomestic containers (Marten 1990, Marten et al. 1994, Schreiber et al. 1996). For example, *Macrocyclops albidus* (Cocal), *Mesocyclops longisetus* (Thiébaund), and *Mesocyclops aspericornis* (Daday) are highly effective in controlling *Aedes albopictus* (Skuse) (Marten 1990) and *Ae. aegypti* larvae in abandoned tires (Suárez et al. 1991, Marten et al. 1993, Marten et al. 1994) and in other artificial habitats (e.g., barrels, water tanks). In other studies, *M. aspericornis* eliminated 99% of the 1st-stage larvae of *Ae. aegypti* and *Aedes polynesiensis* (Marks) in artificial habitats (Riviere et al. 1987; Brown et al. 1991a, 1991b). In addition, copepods may help in controlling mosquito larvae in natural habitats (e.g., tree holes, crab holes) (Lardeaux 1992). *Mesocyclops aspericornis* reduced larval populations of *Ae. polynesiensis* and *Ae. aegypti* by 91–99% (Riviere et al. 1987), demonstrating that the copepods have a potential value as a biological control agent (Kay et al. 1992).

In Cali, Colombia, the principal breeding sites of *Ae. aegypti* are catch basins (Fig. 1), located along streets in residential areas. Catch basins have been described as a primary breeding site in Cali because *Ae. aegypti* larvae were present in 57.2% of the city's basins (González et al. 1993). Due to their design, the basins contain water throughout the year, thus providing appropriate conditions for the development of mosquito larvae. Additionally, catch basins in Cali produce 27 times more pupae than all other breeding sites combined within a city block (González and Suárez, unpublished data), suggesting that catch basins are mosquito factories.

The characteristics of catch basins make it difficult to incorporate traditional control strategies that are effective in other places. For example, chemical control, especially liquid or granular formulations, require continued applications to control

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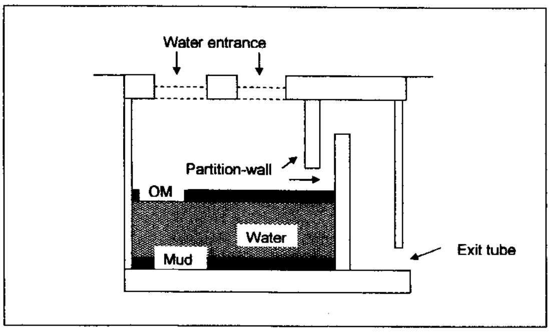

Fig. 1. Schematic drawing of catch basins. The basins constantly contain standing water, and they have various amounts of organic matter (OM).

mosquito populations because its effectiveness lasts for 8–15 days. Slow-release formulations are more expensive and the government does not support them, and they may cause environmental damage (Schreiber et al. 1996). Additionally, *Ae. aegypti* populations in Cali are resistant to temephos, one of the most commonly used larvicide for their control (González et al. 1993). Thus, it became necessary to evaluate biological control as an alternative to the chemical control of *Ae. aegypti.*

This article presents data on the efficacy of *M. longisetus* as a biocontrol agent in controlling *Ae. aegypti* larvae in catch basins and the appropriate characteristics of catch basins for its establishment. We hypothesized that, after the introduction of *M. longisetus,* the proportion of catch basins with *Ae. aegypti* larvae would be reduced, whereas the proportion of catch basins with copepods would increase.

MATERIALS AND METHODS

Study site

We evaluated 201 catch basins along streets in residential areas in southern sectors of Cali (3°27′N, 76°32′W), Colombia (elevation 995 m). The annual precipitation is 1,153 mm, with rainy peaks in April and October, and dry peaks in July and December, and the average temperature is 23°C (IGAC 1980).

Field surveys

Between June 1999 and February 2000, 201 catch basins were evaluated on a monthly basis for presence and absence of *M. longisetus* and *Ae. aegypti* larvae and 70 catch basins were used as controls. In June, we sampled the *Ae. aegypti* larvae in the catch basins to define the proportion of infested basins. In July, we introduced approximately 500 adult copepods per basin. The copepods were taken from laboratory culture and counted using a plastic dropper. In August, an 8-month survey began to determine the proportion of basins with and without copepods and *Ae. aegypti* larvae. In each visit, 4 samples of water were taken from each basin using a 200-cm^3 ladle. The samples were taken from each corner of the basin at 5-min intervals to allow the larvae and the copepods to return to the organic matter, and each sample was considered individually for further analysis. In addition, to identify the characteristics correlated with copepod establishment in the basins, we evaluated 3 physical features of catch basins (i.e., percentage shade, percentage cover of floating organic matter, and the topography around the basin). The topography was measured as a function of the inclination of the road where the basin was located. Finally, the presence of any synthetic washing agent, such as detergent, inside catch basins was recorded.

Statistical analysis

Chi-square tests were performed to evaluate differences in presence of *Ae. aegypti* larvae and copepods in catch basins over time. The corrected efficacy percent was calculated using the Henderson–Tilton's formula (Henderson and Tilton 1955). A principal component analysis (PCA) was performed to analyze the effect of the percent shade, percent cover of organic matter, and the topography on copepods establishment. Afterward, multiresponse permutation procedures (MRPP) were used to determine if there were statistical differences between the groups in the PCA. Moreover, an association test was used to determine differences in copepod presence between catch basins with and without detergent. The level of significance for all tests was 0.05.

RESULTS

The number of catch basins infested with *Ae. aegypti* larvae significantly decreased after copepods were introduced ($\chi^2 = 36.48$, df = 1, $P < 0.000$), but the number of catch basins with copepods did not differ through time ($\chi^2 = 0.3368$, df = 1, $P = 0.562$). The number of catch basins with larvae decreased significantly over time, but the basins where copepods established remained positive for copepods (49.2%), and in these basins, the copepods were present for 8 months (Fig. 2). The corrected efficacy percent was 90.4% and the mean of larvae density per sample was 0.53.

From the 201 catch basins, 68% were located in places in which they were exposed to sunlight, 87% were located in roads with low slope, and 91% received less than 50% of floating organic matter (Fig. 3). The amount of shade and the nature of local topography were the most important features contributing to the establishment of copepod populations. In the PCA, axis 1 was correlated with shade and topography, explaining 48.6% of the variance with an eigenvalue of 1.458; while axis 2 was

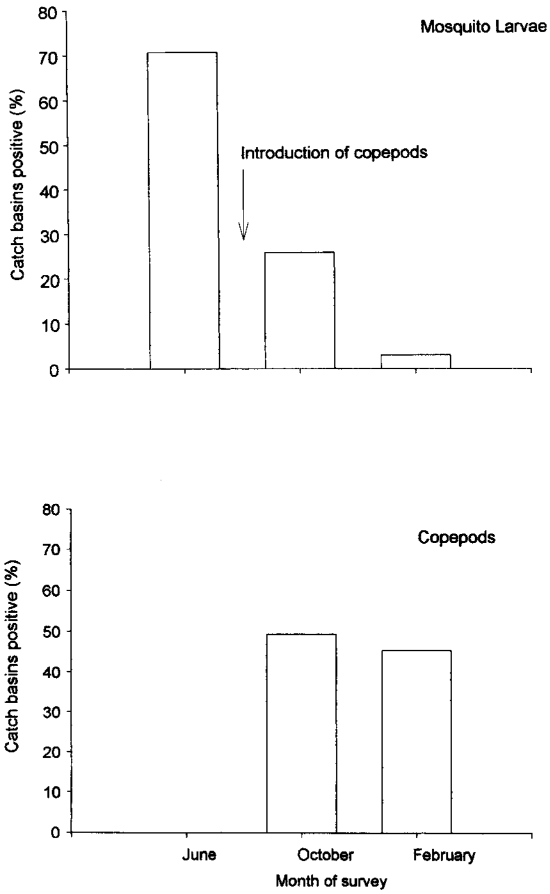

Fig. 2. (a) Proportion of catch basins infested with *Aedes aegypti* larvae before and after the introduction of the copepod *Mesocyclops longisetus,* and (b) proportion of catch basins with established *M. longisetus* populations.

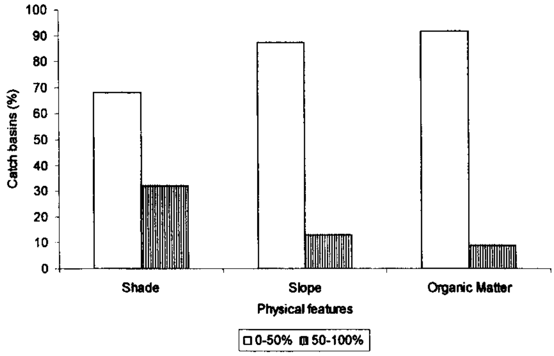

Fig. 3. Proportion of catch basins characterized by 3 physical features (i.e., shade, slope, and floating organic matter).

correlated with organic matter, explaining 32.6% of the variance with an eigenvalue of 0.977. The copepods did not establish in catch basins located in areas with steep slopes and under complete shade. However, they did establish in basins located in flat areas, exposed to sunlight, and with 0–10% of organic matter. The MRPP found significant differences between these two groups ($T = -12.38$, $P = 0.000$). Nevertheless, copepods were absent from 20% of catch basins with suitable characteristics if detergent that came from drainage of the neighborhood was present (Yate's corrected $\chi^2 = 8.83$, $P = 0.003$).

DISCUSSION

After 8 months, the percent of catch basins infested with *Ae. aegypti* larvae dropped from 71 to 3%. These data demonstrate the well-known predatory activity of the copepods (Marten et al. 1994). As long as the copepod populations are established and prevailing in the catch basins, they are able to suppress *Ae. aegypti* larvae. Our results are in agreement with those of Rawlins et al. (1997), who found that, in Trinidad, *M. longisetus* was effective as a biological control tool against *Ae. aegypti* larvae because the copepods almost completely suppressed the larvae in drums and tires. However, *M. longisetus* showed low predatory capacity against *Culex quinquefasciatus* (Say) because *M. longisetus* is benthic and preys on bottom-feeding *Ae. aegypti* but have little effect in reducing surface-feeding *Cx. quinquefasciatus* populations (Riviere et al. 1987). In contrast, Kay et al. (1992) found that larger individuals of *M. longisetus* were effective predators of both *Ae. aegypti* and *Cx. quinquefasciatus* larvae in the field.

The number of catch basins positive for *M. longisetus* in Cali did not increase throughout time. It successfully got established in 49.2% of the catch basins and maintained its numbers during the study period (8 months) in these basins. The survival of copepods in catch basins is consistent with results of studies in Honduras, in which 79% of the sampled drums had copepods 7 months after their introduction (Marten et al. 1994). The difference in the magnitude of copepods established in Honduras and Cali could be due to the characteristics of the artificial containers. Drums are used to store water and are exposed to continuous sunlight, while catch basins may be in shaded areas but may flush during rains if they are located in areas with steep slopes. Because the copepods feed on algae and protozoa (Suárez et al. 1992), the sunlight allows the fast growth of these photosynthetic groups. It was noted that the copepods do not tolerate water contaminated with detergents, possibly due to the toxic action that kills the copepods but did not kill the *Ae. aegypti* larvae. Thus, copepods should not be used in catch basins where the drainage may be contaminated with any synthetic washing agent.

The copepod *M. longisetus* is a good candidate as a biological control agent because it serves as a sustainable control strategy. It suppresses *Ae. aegypti* populations and tolerates organophosphate insecticides used in mosquito control (Tietze et al. 1994, Rawlins et al. 1997). Additionally, *M. longisetus* thrives in the presence of *Bacillus thuringiensis* var. *israeliensis* (*Bti*), a commonly used microbiological insecticide (Riviere et al. 1987, Marten et al. 1993). Thus, application of *Bti* to the habitat of this copepod does not impact copepods adversely. However, there are some disadvantages in using *M. longisetus* as a biological control agent. One major disadvantage is that, after introduction, the predatory pressure on mosquito larvae lags until the copepods reach high population densities (Tietze et al. 1994). In addition, they cannot survive desiccation (Riviere et al. 1987) and detergents. Hence, the disadvantages may be solved if copepods are used along with a safe microbial control agent and in catch basins without inflow of detergents. Because of their short life cycle, their high fecundity, their protozoa-based diet, and their tolerance to *Bti, M. longisetus* could be incorporated as a biological control agent in an integrated control program of *Ae. aegypti.*

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REFERENCES CITED

Brown M, Kay B, Hendrikz J. 1991a. Evaluation of Australian *Mesocyclops* (Cyclopoida: Cyclopidae) for mosquito control. *J Med Entomol* 28:618–623.

Brown MD, Kay BH, Greenwood JG. 1991b. The predation efficiency of north-eastern Australian *Mesocyclops* (Copepoda: Cyclopoida) on mosquito larvae. *Bull Plankton Soc Japan* Special:329–338.

Clark GG. 1995. Situación epidemiológica del dengue en América. Desafíos para su vigilancia y control. *Salud Pública de México* 37:S5–S11.

González R, Gamboa F, Méndez O. 1993. *Índices de positividad y asociación de* Aedes aegypti *(Diptera: Culicidae) en sumideros de la ciudad de Santiago de Cali* [Abstract]. V Congreso Latinoamericano y XIII Venezolano de Entomología. July 4–8, 1993; Porlamar, Venezuela. Sociedad Venezolana de Entomología. p 159–160.

Gratz NG, Knudsen AB (World Health Organization). 1995. *The rise and spread of dengue, dengue haemorrhagic fever and its vectors. A historical review (up to 1995).* Geneva, Switzerland: WHO/CTD/FIL (DEN) 96.7.

Gubler DJ, Clark GG. 1996. Community involvement in the control of *Aedes aegypti. Acta Tropica* 61:169–179.

Halstead SB. 1984. Dengue. *Rev Infect Dis* 6:275–287.

Henderson CF, Tilton EW. 1955. Tests with acaricides against the brown wheat mite. *J Econ Entomol* 48:157–161.

Howarth FG. 1991. Environmental impacts of classical biological control. *Annu Rev Entomol* 36:485–509.

IGAC. 1980. *Diccionario Geográfico de Colombia. Tomo I.* Segunda Edición. Bogotá, Colombia: Instituto Geográfico Agustín Codazzi.

Kay BH, Cabral CP, Sleigh AC, Brown MD, Ribeiro ZM, Vasconcelos AW. 1992. Laboratory evaluation of Brazilian *Mesocyclops* (Copepoda: Cyclopidae) for mosquito control. *J Med Entomol* 29:599–602.

Laird M. 1981. *Biocontrol of medical and veterinary Pests.* New York: Praeger.

Lardeux F. 1992. Biological control of Culicidae with the copepod *Mesocyclops aspericornis* and larvivorous fish (Poeciliidae) in a village of French Polynesia. *J Med Entomol* 6:9–15.

Leontsini E, Gil E, Kendall C, Clark GG. 1993. Effect of a community-based *Aedes aegypti* control program on mosquito larval production sites in El Progreso, Honduras. *Trans Roy Soc Trop Med Hyg* 87:267–271.

Marten GG. 1990. Evaluation of cyclopoid copepods for *Aedes albopictus* control in tires. *J Am Mosq Control Assoc* 6:681–693.

Marten GG, Borjas G, Cush M, Fernández E, Reid J. 1994. Control of larval *Aedes aegypti* (Diptera: Culicidae) by cyclopoid copepods in peridomestic breeding containers. *J Med Entomol* 31:36–44.

Marten GG, Che W, Bordes ES. 1993. Compatibility of cyclopoid copepods with mosquito insecticides. *J Am Mosq Control Assoc* 9:150–154.

Nelson MJ (Pan American Health Organization). 1986. Aedes aegypti*: Biology and ecology.* Washington, DC: PAHO PNSP/86–64.

PAHO [Pan American Health Organization]. 1995. *Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control.* Geneva, Switzerland: Scientific publication 548.

Rawlins SC, Martínez R, Wiltshire S, Clarke D, Prabhakar P, Spinks M. 1997. Evaluation of Caribbean strains of *Macrocyclops* and *Mesocyclops* (Cyclopoida: Cyclopidae) as biological control tools for the dengue vector *Aedes aegypti. J Am Mosq Control Assoc* 13:18–23.

Riviere FB, Kay H, Klein JM, Sechan Y. 1987. *Mesocyclops aspericornis* (Copepoda) and *Bacillus thuringiensis* var. *israeliensis* for the biological control of *Aedes* and *Culex* vectors (Diptera: Culicidae) breeding in crab holes, tree holes, and artificial containers. *J Med Entomol* 24:425–430.

Schreiber ET, Hallmon CF, Eskridge KM, Marten GG. 1996. Effects of *Mesocyclops longisetus* (Copepoda: Cyclopoidae) on mosquitoes that inhabit tires: influence of litter type, quality, and quantity. *J Am Mosq Control Assoc* 12:688–694.

Suárez MF, Marten G, Clark GG. 1992. A simple method for cultivating freshwater copepods used in biological control of *Aedes aegypti. J Am Mosq Control Assoc* 8: 409–412.

Suárez MF, Rivera MM, Aranda C, Clark GG, Marten G. 1991. *Aedes aegypti* larval control using cyclops in Puerto Rico. *J Am Mosq Control Assoc* 7:640–641.

Tietze NS, Hester PG, Shaffer KR, Prescott SJ, Scheiber ET. 1994. Integrated management of waste tire mosquitoes utilizing *Mesocyclops longisetus* (Cyclopoida: Cyclopidae), *Bacillus thuringiensis* var. *israeliensis, Bacillus sphaericus,* and methoprene. *J Am Mosq Control Assoc* 10:363–373.