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EVALUATION OF CARIBBEAN STRAINS OF *MACROCYCLOPS* AND *MESOCYCLOPS* (CYCLOPOIDA: CYCLOPIDAE) AS BIOLOGICAL CONTROL TOOLS FOR THE DENGUE VECTOR *AEDES AEGYPTI*

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ABSTRACT. Fifteen Caribbean strains of copepods were assessed for their predation ability against mosquito larvae. *Macrocyclops albidus* from Nariva, *Mesocyclops aspericornis* from Oropouche, and *Mesocyclops longisetus* from El Socorro, Trinidad, were most effective against *Aedes aegypti* but not against *Culex quinquefasciatus*. *Mesocyclops longisetus* and *Me. aspericornis* prevented any mosquito survival over 25 wk of observation despite weekly challenges with *Ae. aegypti*. The copepods were tolerant to dosages of the insecticide temephos that are usually toxic to mosquito larvae. This indicated that copepods could be incorporated into an integrated control system. To determine whether pathogenic microbes might be introduced with copepods into drinking water, microbial studies were done on the copepods. These showed the presence of only *Aeromonas sobria*, *Pseudomonas* sp., *Alcalignes* sp., and gram-positive bacilli. Although none of these are highly pathogenic to humans, the application of these copepods has not yet been recommended for use in drinking water.

INTRODUCTION

The continuing endemicity of dengue serotypes 1, 2, and 4 in the Caribbean region together with the risk of introduction of dengue type 3 from Central America has greatly emphasized the need for efficient management of the vector *Aedes aegypti* (Linnaeus) (Nathan 1993, Pan American Health Organization 1994). There is evidence of failure of source reduction practices for controlling this container breeder due to the reluctance of some Caribbean householders to discard most potential mosquito-producing containers (Rosenbaum et al. 1995). Also, the development of insecticide resistance in several Caribbean populations of *Ae. aegypti* (Rawlins and Hing Wan 1995) has forced mosquito control authorities to seek alternative sustainable systems for management of this mosquito.

Biological control tools such as the mosquito *Toxorhynchites moctezuma* (Dyar and Knab) have been evaluated for management of *Ae. aegypti* (Rawlins et al. 1991, Tikasingh 1992). This has met with only minimal success due to failure of the predator to find and colonize habitats of its prey in urban environments where *Ae. aegypti* is known to thrive.

Recently, certain cyclopoid copepods have proven to be capable of controlling *Ae. aegypti* larvae in peridomestic breeding containers (Marten 1990, Marten et al. 1994). Marten et al. (1992) reported that one species, *Mesocyclops longisetus* (Thiébaud), was capable of reducing *Ae. aegypti* larval populations by more than 99.9%.

Other authors have evaluated some North American copepods against container-breeding mosquitoes such as *Aedes albopictus* (Skuse) (Schreiber et al. 1993). Brazilian strains of *Mesocyclops aspericornis* (Daday) showed potential as biological control agents against *Ae. aegypti* but were not as effective against *Anopheles* and *Culex* (Kay et. al. 1992). In contrast, the larger *M. longisetus* killed 100% of *Ae. aegypti*, *Anopheles farauti* Laveran, and *Culex quinquefasciatus* Say.

Vasconcelos et al. (1992) in small-scale field trials in Brazil found that *M. longisetus* controlled *Ae. aegypti* in containers that produced more than 80% of the mosquito. They found the involvement of the community in such a project to be very helpful. In Mexico, *M. longisetus* selected from 15 species of copepods collected in Nuevo Leon and Coahila was equally predaceous on both *Ae. aegypti* and *Cx. quinquefasciatus* (Rodriguez 1992). In the Caribbean islands of Puerto Rico and Anguilla, *M. aspericornis* controlled but did not eliminate *Ae. aegypti* in drum habitats (Suarez 1992).

The prospect of incorporating copepods into an integrated control system for mosquitoes has attracted the attention of several mosquito control workers. Thus, the combination of copepods with *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*), *Bacillus sphaericus*, and methoprene (Tietze et al. 1994), or with insecticides (Marten et al. 1993), has improved the performance of various copepod species.

This paper evaluates the larval consumption characteristics of a number of Caribbean copepod taxa and their capability of surviving in insecticide-treated habitats and their potential for incorporation into an integrated control program for *Ae. aegypti*. The present study provides data from such work performed in Port-of-Spain, Trinidad.

MATERIALS AND METHODS

Collection of copepods: Various copepod samples were collected from freshwater bodies in different Caribbean locations. The copepods were taken to the entomological laboratories at the Caribbean Epidemiology Centre (CAREC; Port-of-Spain, Trinidad) and were reared on a diet of *Par-*

amecium caudatum, *Chilomonas* sp. (Suarez et al. 1992), and 1st-instar mosquito larvae. For each sample a culture was started from a single gravid female. The copepods were maintained under laboratory conditions at ambient temperatures of 21–34°C until they were evaluated for their predatory ability against 1st-instar mosquito larvae. Copepod samples from each strain were sent to Janet Reid at the Smithsonian Institution, Washington, DC, who kindly verified the species identifications. A strain of *M. longisetus curvatus* (Dussart) was also received from M. E. Suarez, CDC, San Juan Laboratories, Puerto Rico, and was used as a reference predatory strain.

Predation studies: First-instar *Ae. aegypti* larvae were introduced into each well of a tissue culture plate each containing one adult copepod in 10 ml of water. Six replicates were done for each strain/species. After 24 h, the percent kill was assessed by removing and counting the number of live and dead larvae, and a new set of 1st-instar larvae was exposed to the copepods. This process of evaluation was repeated 5 times and the mean percent mortality of *Ae. aegypti* larvae for each copepod strain/species was assessed.

Insecticide susceptibility of copepods: Preliminary laboratory testing was conducted on 4 strains of copepods: *Macrocyclops albidus principalis* (Herbst) (Nariva), *M. longisetus* (CDC), *M. longisetus* (El Socorro), and *M. aspericornis* (Daday) (Oropouche). From each strain, 10 copepods were placed into their respective wells of a tissue culture plate with 10 ml of solution containing varying concentrations of temephos. The 6 concentrations chosen ranged from 0.02 mg/liter (the diagnostic dosage for *Ae. aegypti* larvae) to 0.22 mg/liter. Mortality was determined by the presence of moribund or dead copepods at 24-h intervals over a 2-wk period. No food was offered to the copepods in both the controls and test wells during this period.

Copepod/temephos integrated control trials: Two of the more predaceous species of copepods, *Mesocyclops* sp. B (near *aspericornis*) from Chaguaramas and *M. aspericornis* from North Oropouche, Trinidad, were selected for this trial. *Mesocyclops longisetus curvatus* obtained from CDC in Puerto Rico was used for comparison to the 2 Trinidad strains of copepods.

Twenty-four drums each containing 200 liters of tap water were allowed to stand covered for 7 days before any additions were made to them. For each strain of copepod, drums were prepared as follows: 1) no treatment, controls (3 drums); 2) 8 g of Abate® (Cyanamid Intl., Wayne, NJ) 1% sand granules (0.4 g temephos) (3 drums); 3) 100 adult copepods alone (9 drums) (3 drums per species); and 4) 100 adult copepods plus 8 g of Abate 1% sand granules (0.4 g temephos) (9 drums) (3 drums per species).

There were a total of 24 drum habitats. To each of these 100 1st-instar *Ae. aegypti* larvae were added and the drums were covered with a fine mesh to prevent entrance of any gravid mosquitoes. The drums were left under partially shaded conditions. At 6-day intervals, all larvae and/or pupae were removed from each drum by very careful sweeping with a fine nylon dip net and emptied into enamel trays. Larvae and/or pupae present were counted, removed, recorded, and the water containing the copepods and/or temephos was returned to its respective drum. A new set of 100 1st-instar *Ae. aegypti* larvae was added to each drum and surviving larvae, if any, were harvested after 6 days. This process was continued for 25 wk.

Field studies in tire habitats: Discarded automobile tires were selected for this field study in St. James, Port-of-Spain, Trinidad, with a known presence of both *Ae. aegypti* and *Cx. quinquefasciatus*. Tires were each treated with one of the following regimes: 1) tire 1, 100 *M. longisetus* in 2 liters of water; 2) tire 2, 200 *M. longisetus* in 2 liters of water; 3) tire 3, 100 *M. aspericornis* in 2 liters of water; 4) tire 4, 200 *M. aspericornis* in 2 liters of water, and 5) tire 5, untreated control, 2 liters of water.

Nine different sites were used for each treatment. The tires were left in shaded locations without protection from gravid wild mosquitoes for 14 wk. The water in the tires was examined by emptying the contents into a white enamel bowl and any mosquito larvae present were removed with a pipette and taken to the laboratory. This was done at 4-day intervals because longer periods could permit pupation and a possible risk of release of adult mosquitoes. The uncounted copepods were returned to their respective tires and the water brought back to its original level. In the laboratory, the larvae for each mosquito species were counted and recorded for each tire habitat.

Statistical treatment: The data were entered using the EPI-Info version 6 program provided by the CDC of the U.S. Public Health Service (Division of Surveillance and Epidemiologic Studies, Epidemiology Program Office, CDC, Atlanta, GA). A repeated multivariate analysis of variance was used to compare regimes, with weeks being selected as the repeated factor. Verification of the assumptions for these statistical tests was made through residual analysis. In cases where the assumptions were not satisfied, a square root transformation was utilized and the test repeated. The usual F statistic was the criterion applied for assessment of group comparisons. Unless otherwise mentioned, the P value of 5% was used as the determinant level of significance. The STAT module of the Statistical Analysis Software (SAS) Version 6 for Windows (SAS Institute, Inc., SAS Campus Drive, Cary, NC) was used for analyzing the data.

Bacteriological analysis of copepods: Bacteriological assays were conducted on 2 species of copepods to determine whether there was a risk of

Table 1. Selective predation of copepods (*Macrocyclops* and *Mesocyclops*) on 1-instar *Aedes aegypti* and *Culex quinquefasciatus* larvae.[1]

Taxa	Mean % predation	
	Aedes	Culex
Macrocyclops albidus (Nariva)	85.7	63.1
Mesocyclops aspericornis (Oropouche)	83.5	53.7
Mesocyclops longisetus (El Socorro)	78.1	58.9
Mesocyclops near *aspericornis* (Chaguaramas)	75.7	45.7
Mesocyclops ellipticus (BBF)	79.0	41.6
Mesocyclops sp. (Arena)	77.8	42.8
Mesocyclops near *longisetus* (AWF)	59.7	46.4
Mesocyclops longisetus (CDC)	72.5	43.1
M. aspericornis (Dominica)	73.9	37.4
Mesocyclops sp. (Antigua)	71.1	34.7
Mesocyclops sp. (Mucurapo ground pool)	45.8	32.0
Mesocyclops sp. (Mucurapo stream pool)	21.2	10.6
Mesocyclops sp. (Otoire)	7.4	3.8

[1] Control mortalities were: *Aedes* alone, 0%; *Aedes* and *Culex*, 0.6% and 5.4%, respectively; *Culex* alone, 5.7%.

introducing pathogenic bacteria into water storage containers with the copepods. The copepods, *M. longisetus* and *M. aspericornis*, from CAREC indoor and outdoor cultures were strained and about 30 individuals from each group were homogenized in sterile containers. The homogenates and water samples from these 2 cultures were analyzed for bacterial agents by the methods described in Lennette (1985).

RESULTS

Copeod taxa collected in Caribbean countries: Some 15 taxa of copeopds were collected from various Caribbean territories. These consisted of 3 strains of *Mesocyclops* sp. A (near *ellipticus*) from Tobago and Trinidad, and *Mesocyclops* sp. B (near *aspericornis*) and sp. C (near *longisetus*) from Trinidad. There were 4 samples of *M. aspericornis* from Trinidad and Dominica, one sample of *M. longisetus curvatus* from El Socorro, Trinidad, and *M. albidus principalis* from Nariva Swamp, Trinidad. In addition, there was one sample each of *Paracyclops chiltoni* (Thompson), *Paracyclops* sp. A, *Elaphoidella* sp. A from Trinidad, and *Allocyclops* sp. from Tobago.

The comparative predatory ability of the 13 strains of copepods against 1st-instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* is shown in Table 1. *Macrocyclops albidus* collected from Nariva Swamp, Trinidad, was the most predaceous strain against *Ae. aegypti* (85.7%). *Mesocyclops aspericornis* from North Oropouche (83.5%) and *M. longisetus* from El Socorro, Trinidad, (78.1%) were also very strong predators, comparing well with the reference *M. longisetus* CDC strain (72.5%). Generally, predation against *Cx. quinquefasciatus* was of a lower order by all the copepod strains/species, ranging from 3.8 to 63.1%.

The 4 strains of copeods showed markedly different insecticide susceptibilities to the larvicide temephos. The results were *M. albidus* (Nariva) 6% (lowest mortality) < *M. longisetus* (CDC) 12% < *M. aspericornis* (Oropouche) 47.3% < < *M. longisetus* (El Socorro) 73.1% (highest mortality).

The comparisons of larval *Ae. aegypti* survival in drums treated with the insecticide temephos, copepods, temephos and copepods combined, or untreated controls are shown in Fig. 1. For the copepod *Mesocyclops* sp. near *aspericornis* (Fig. 1A) the copepod alone demonstrated control of *Ae. aegypti*. Between weeks 3 and 18, containers with copepods alone kept the mosquito production between 2 and 18% of the weekly introduced larvae.

Copepods of the *M. aspericornis* (Oropouche) and *M. longisetus* (CDC) strains maintained their mosquito larval population at 0% survival throughout the 25 wk of the study (Figs. 1B, 1C). Control (untreated) drums were constantly productive, producing between 95 and 98% of weekly introduced (1st-instar) larvae. Drums treated with temephos alone were free of mosquitoes initially but began to lose their efficacy after week 17, and by week 25 were as productive as the control containers. Drums treated with *M. aspericornis* (Oropouche) plus temephos and *M. longisetus* (CDC) plus temephos were both very effective against *Ae. aegypti* production (Figs. 1B, 1C), but *Mesocyclops* sp. near *aspericornis* was effective to a lesser degree (Fig. 1A). At the end of the 25-wk study, copepods were present in all drums into which they had been introduced, but they were not quantified.

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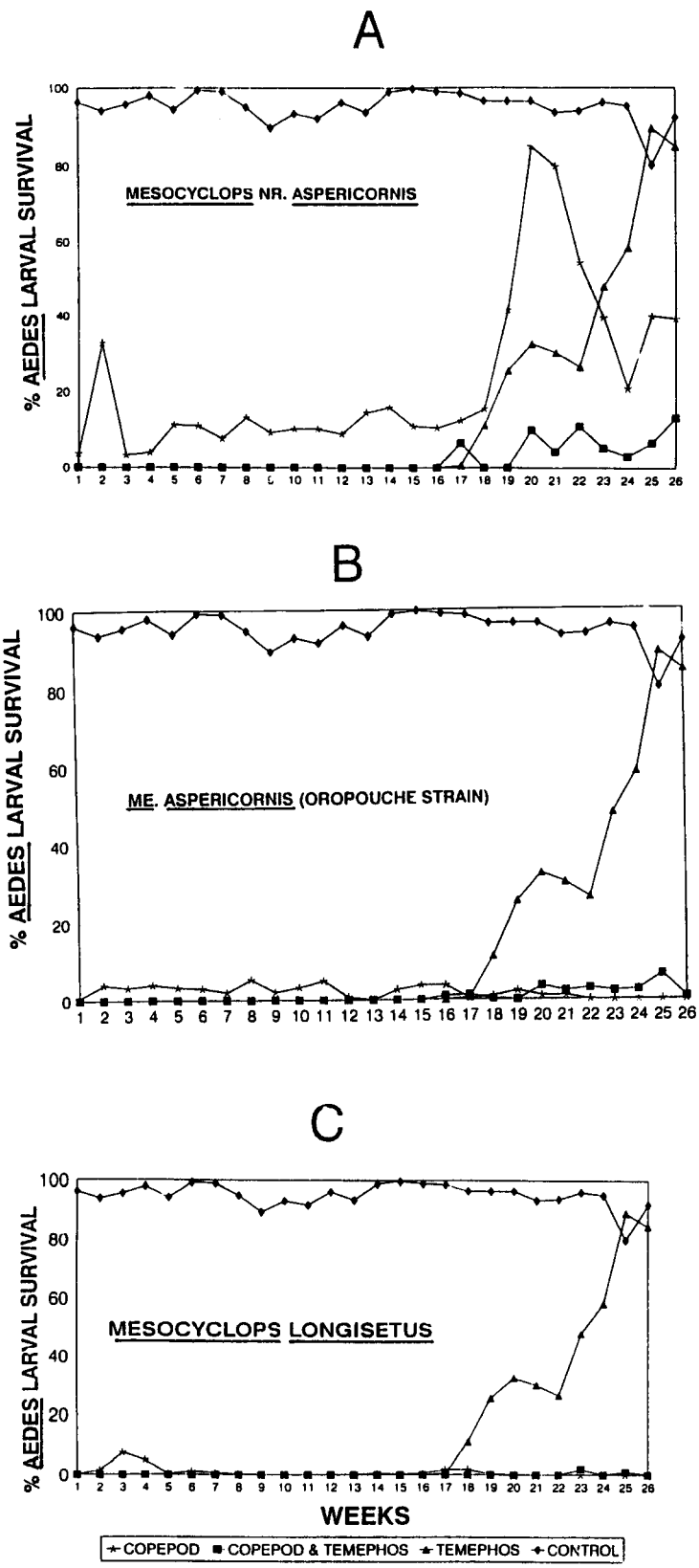
Fig. 1. Weekly survival of *Aedes aegypti* larvae in drums treated with copepods and/or temephos. A. *Mesocyclops* near *aspericornis*. B. *Mesocyclops aspericornis* (Oropouche strain). C. *Mesocyclops longisetosus*.


A
% AEDES LARVAL SURVIVAL
MESOCYCLOPS NR. ASPERICORNIS
B
% AEDES LARVAL SURVIVAL
ME. ASPERICORNIS (OROPOUCHE STRAIN)
C
% AEDES LARVAL SURVIVAL
MESOCYCLOPS LONGISETUS
WEEKS
COPEPOD
COPEPOD & TEMEPHOS
TEMEPHOS
CONTROL

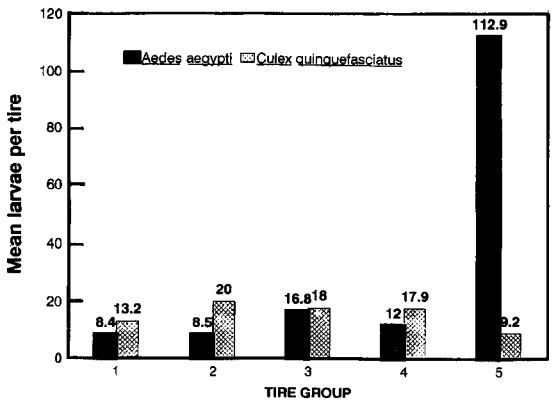


Fig. 2. Mosquito larvae found in tire habitats previously treated with copepods.

The *Ae. aegypti* larval production in tire habitats in the St. James area is shown in Fig. 2. The presence of either species of copepod (*M. longisetus* or *M. aspericornis*) seeded with 100 or 200 copepods in 2 liters of water made a significant ($F = 6.95$, $df = 8$, $P = 0.0001$) difference for *Ae. aegypti* larval survival in tires when they were compared to the control (untreated tires). Means were 8.4 and 8.5 mature larvae per tire in *M. longisetus*-treated tires and 16.8 and 12.0 larvae per tire in *M. aspericornis*-treated tires compared to 112.9 larvae per tire in the control tires. There were no significant differences between the 2 copepod species or dosage treatments ($F = 2.44$, $df = 1$, $P = 0.12$). There were no differences in production of *Cx. quinquefasciatus* based on species of copepod or initial dosage of copepod per habitat (Fig. 2). In fact, the control habitats were consistently lower producers of this mosquito than the habitats treated with copepods.

Microbial studies done on *M. longisetus* maintained indoor and outdoor at CAREC showed the presence of *Aeromonas sobria*, *Enterobacter* sp., and *Pseudomonas* sp. *Mesocyclops aspericornis* homogenates grew *A. sobria*, *Alcalignes* sp., gram-positive bacilli, gram-positive cocci, and *Pseudomonas pseudoalcalignes*. Watery media from *M. longisetus* culture produced gram-positive rods and cocci as well as *P. pseudoalcalignes* and gram-negative rods.

DISCUSSION

The availability of mosquito-larvivorous copepods in the Caribbean region offer a good promise for control of *Ae. aegypti* now that appropriate strains of *Macrocyclops* and *Mesocyclops* have been identified and assayed. The 84–86% control of *Ae. aegypti* by *M. aspericornis* (Oropouche) and *M. albidus* are reminiscent of the 99.9% control of *Ae. aegypti* by *M. longisetus* (Marten et al. 1992). Our results are also similar to those of other workers, for example, Vasconcelos et al. (1992) in Brazil and Rodriguez (1992) in Mexico, who assayed *Mesocyclops* and found them to be efficacious as a biocontrol tool against *Ae. aegypti*.

Macrocyclops albidus (Cocal), *M. aspericornis* (Oropouche), and the reference species *M. longisetus* exhibited tolerance to the organophosphorous insecticide temephos. This tolerance suggests the validity of incorporating insecticide-tolerant copepods as part of an integrated control system for *Ae. aegypti*: accidental or intended insecticide contamination will not necessarily eliminate the copepod population. Also, a complete change over from temephos to copepod biocontrol would not present many problems. These strains of copepods, by not being harmed by temephos, showed similarity to *Macrocyclops*, *Mesocyclops*, and *Acanthocyclops* sp. that thrived in the presence of *B.t.i.* or permethrin (Riviere et al. 1987, Marten et al. 1993) or of *B.t.i.* and methoprene (Tietze et al. 1994).

Drums and tires are among the most common and most productive containers for *Ae. aegypti* in Trinidad and Tobago (Rosenbaum et al. 1995). The present data show that although copepods are useful tools against *Ae. aegypti* in these 2 types of containers, copepods only showed low to moderate performance (3.8–63.1% control) against *Cx. quinquefasciatus*. In this respect our results appear to agree with Riviere and Thirel (1981) and Kay et al. (1992), who found that *Cx. quinquefasciatus* were not greatly affected by *Mesocyclops leukarti pilosa* (Daday) and *M. aspericornis*, respectively. Kay et al. (1992) did find, however, that the larger *M. longisetus* was efficacious against *Ae. aegypti*, *An. farauti*, and *Cx. quinquefasciatus*.

The bacteria isolated from *M. longisetus* and *M. aspericornis*, whether maintained indoors or outdoors or from their watery medium, are generally regarded as commensals in humans. We do not know whether these bacteria were located internally or externally on the chitin of the copepods. Members of the Enterobacteriaceae are widely distributed in soil and plants as well as being normal colonizers of the intestinal tract of humans and animals. The major intestinal tract pathogens such as *Salmonella*, *Shigella*, and *Vibrio cholerae*, which has a particular affinity for chitin (Nalin et al. 1979), were not found in our samples. However, *Aeromonas* spp., which are ubiquitous inhabitants of water and soil, may cause disease in amphibians, reptiles, and fish; *A. sobria* has been associated with extraintestinal infections in humans (Janda and Brenden 1987). However, the risk of such infection is minimal in the utilization of water treated with copepods. Despite this, the use of copepods for *Ae. aegypti* control is currently being recommended for non-drinking water containers only.

The present work shows that there are several Caribbean strains of copepods capable of being incorporated alone or in association with organophosphorous insecticides for *Ae. aegypti* control

and thus dengue management. These copepod strains could be mass-produced in the various Caribbean countries, and even by local communities, and distributed within neighborhoods for *Ae. aegypti* control programs, as was suggested by Marten et al. (1992) in Honduras. Such use of copepods in control programs could be achieved with appropriate training and education. Vasconcelos et al. (1992) found that in Brazil many residents, especially school children, became very interested and participated in copepod production and use. Once the trained community has a part in copepod production and distribution, the use of the biocontrol tool could become a cheap, sustainable, and efficacious *Ae. aegypti* management system for the Caribbean.

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