OPERATIONAL AND SCIENTIFIC NOTES

A SIMPLE METHOD FOR CULTIVATING FRESHWATER COPEPODS USED IN BIOLOGICAL CONTROL OF *AEDES AEGYPTI*

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ABSTRACT. A simple method for indoor and outdoor cultivation of *Mesocyclops aspericornis*, *Macrocyclops albidus* and *Mesocyclops* n. sp. copepods is presented. This method utilizes *Chilomonas* sp., *Paramecium caudatum* and fresh lettuce as food sources for copepod cultures. Steps for initiating and maintaining copepod cultures are provided.

Cyclopoid copepods (planktonic microcrustaceans) have been identified as a promising new form of biological control for container-breeding mosquito species such as *Aedes aegypti* (Linn.). Three species, *Mesocyclops aspericornis* (Daday), *Me. longisetus* (Thiébaud) and *Macrocyclops albidus* (Jurine), have been most frequently studied (Rivière et al. 1987, Marten 1990, Brown et al. 1991). Copepods can be obtained directly from natural sites or artificially cultivated for use in control interventions. At some locations it may be possible to use copepods from natural sites as a large-scale source for supplying control programs, but this procedure involves repetitive collection trips to bodies of water where they occur, and considerable time is spent separating them from other microfauna and organic detritus.

An important requirement of the World Health Organization (WHO) scheme for screening and evaluating the efficacy of biocontrol agents for disease vectors is that the candidate species must be easily cultivated (WHO 1975). In this paper, we present a simplified procedure for isolating and rearing large numbers of freshwater copepods, which we have employed at the Dengue Branch, Centers for Disease Control in San Juan, Puerto Rico during the past 2 years.

We cultivated 3 of the largest species (ca. 1.5 mm in length) of copepods collected in Puerto Rico: *Me. aspericornis*, from a small stream in the municipality of Dorado, which has been in culture since January 1990; *Ma. albidus*, from Lake Carraizo in Trujillo Alto, which has been in culture since April 1990; and *Mesocyclops* n. sp. from a freshwater shrimp production pond in Sabana Grande, which has been in culture since June 1990.

Culture facilities: For indoor cultivation, laboratories should have at least 3 × 2 m of floor space, hot and cold water, a 20-liter sink, and shelves of sufficient size and capacity to support large plastic rearing pans. The culture of tropical copepods is best at temperatures between 24 and 28°C; our laboratory was maintained at a temperature of 26 ± 1°C. Although it is not critical for copepod culture, our laboratory had a 10h:14h, light:dark cycle.

Chlorine-free water is critical for maintaining healthy copepod cultures. Where water is treated, chlorine should be removed before it is used. The chlorine in the San Juan water supply was eliminated by allowing the water to stand at room temperature for 24 h. With some water supplies, it may be necessary to leave the water for several days or longer. Chlorine can be eliminated by adding chemicals (liquids or tablets), which also eliminate heavy metals, such as those used in freshwater aquaria.[4,5] Buffering was not necessary for our water which has a pH of 6.8.

Copepod food: Since copepods attack moving prey in their natural habitat, live organisms are

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[4] The use of trade names or commercial sources is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

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needed as a food source. Previous attempts to culture copepods in the laboratory, mixed algal cultures (*Chlamydomonas* sp. and *Scenedesmus* ssp.) as food for nauplii (i.e., the early developmental stages), and cultures of protozoans of any of the following species *Paramecium caudatum* (Ehrenberg), *P. aurelia* (Ehrenberg) and *P. bursaria* (Ehrenberg and Focke) (Brandl 1973). The microfauna provide the protein necessary for growth and survival of copepodids (i.e., preadults) and adults, and for successful reproduction of the adults (Smyly 1970).

The copepod food regime that we describe is based on a system developed by one of us (GGM), which consisted of *Chilomonas* sp. (a microflagellate), *P. caudatum* and wheat seed. *Chilomonas* sp. served as food for the earlier stages of copepod development (i.e., nauplii and early copepodids). *Paramecium caudatum* was provided for the later stages and wheat seed provided the organic substrate to fuel the food system for copepod cultures. These protozoans can be isolated from freshwater habitats using a standard, but time-consuming, protocol (Galtsoff et al. 1937). Cultures of *P. caudatum* and *Chilomonas* sp. are commercially available.[6] We tested *P. multimicronucleatum* (Power and Michell) but found that *P. caudatum* gave the best results. We sought an alternative to wheat seed because it is often difficult to obtain from commercial sources. One of us (MFS) tested different types of vegetable materials, and found lettuce to be best. Lewis et al. (1971) cautioned against bacterial contamination of copepod cultures, and at outset we boiled the lettuce before using it, but later found this extra step to be unnecessary. No algae are added to this system.

The most important part of a copepod production program is ensuring that an adequate quantity of food is available before the copepod culture is initiated. For our food cultures we used wide mouth Mason jars (900-ml capacity) that had been washed with hot water (at least 60°C) without soap before use. Stock cultures of *P. caudatum* and *Chilomonas* sp. were started by placing 50 ml of inoculum culture in the Mason jar with a 10-cm^2 piece of fresh lettuce, filling it nearly to the top with dechlorinated water, and covering the top with aluminum foil to minimize evaporation and prevent dust or contamination with other microorganisms. It is possible to maintain mixed cultures (1:1) of *P. caudatum* and *Chilomonas* sp. Excess lettuce should be avoided because it can produce anaerobic conditions which are deleterious to the culture of these protozoans.

The density of *P. caudatum* and *Chilomonas* sp. in the cultures should be assessed before using them to feed copepods. Three or 4 days after beginning a culture, a sample from each Mason jar should be examined under a microscope at a magnification of 60×. The culture is ready to use if the protozoan population exceeds 500 specimens per field. To harvest the protozoan culture, the contents of each Mason jar was filtered through a standard tea strainer to remove the lettuce particles. To maintain the stock culture, 50–100 ml of the original culture should be left in the Mason jar to start a new culture. The Mason jar should then be filled with dechlorinated water and a fresh piece of lettuce should be added.

Starting the cultures: Copepods are commonly found in a variety of temporal and permanent aquatic habitats, ranging from groundwater pools and wetlands to lakes and open sea. To collect them, we used a standard plankton net with a 200-ml plastic bottle that could be detached to carry samples to the laboratory. In our studies, we used copepods from freshwater reservoirs and small streams. In the laboratory, we separated larger copepod species from other microorganisms using a medicine dropper under a stereomicroscope. Representative specimens from the initial field collections were preserved in 80% ethyl alcohol for subsequent taxonomic identification.

Cultures should be started with single females because it is common to collect several similar-looking species of copepods from same site. To initiate species monocultures, individual females bearing egg sacs (now we know that it is possible to initiate the culture with individual adults) from each site were isolated in separate 200-ml bowls[6] with 100 ml of the copepod food culture described above. Specimens were removed from each bowl for identification soon after the first generation of progeny matured. After 2 wk, each bowl contained 20–50 adult copepods that could be used to initiate large-scale cultures. Some of the copepods were removed from each bowl for identification at this time. Because copepods must be killed to examine key taxonomic characters, positive species identification of a culture was possible only after there were progeny.

Many types of containers can be used for copepod production. To hold parent cultures of copepods, we used the 20-liter glass or plastic bottles that are used to distribute drinking water. Use of bottles minimizes contamination of parent cultures with other species of copepods or aquatic invertebrates. To start a copepod culture in a bottle, we poured 10 liters of proto-

[6] Carolina Biological Supply Co., 2700 York Road, Burlington, NC 27215.

zoan culture, added a 20-cm^2 piece of fresh lettuce, added fewer than 10 copepods, and covered the top of the bottle with aluminum foil. Between cultures, we cleaned the bottles by pouring in 20 ml of bleach, filling with water, leaving the bleach solution 4 h, rinsing the bottle thoroughly, and using aquarium dechlorination solution for culture water that is immediately placed in the bottle after cleaning.

Mass production: Trays are much better than bottles for mass production because trays provide a large surface area for oxygen exchange. Plastic trays (45 × 35 × 18 cm) that are used to rear mosquito larvae are ideal. The trays should be washed with hot water before use. To start a copepod culture in the tray, pour ca. 2.5 liters (i.e., 3 Mason jars) of a mixture of protozoan cultures into a tray. This will produce a depth of 3 cm in the tray, which is sufficient for copepod cultivation. Add 20–30 adult copepods and a 20-cm^2 piece of lettuce. Cover the tray with aluminum foil to minimize surface evaporation, and place a label on each tray to indicate the species being cultivated and the date that the culture was begun. The copepods should come from a parent culture or another production tray that is known to contain only one species.

It is important not to initiate a culture with too many copepods because overstocking will lead to excessive production of new copepods and the food supply will be exhausted before the culture matures. There is no need to worry about mixing males and females when stocking a copepod culture. The great majority of adult copepod stocks consist almost entirely of females that have been inseminated for life. The females will continue to produce multiple batches of egg sacs (and nauplii) about every 5 days as long as they have food. Females will live and reproduce for several months.

The new generation of copepods in a culture should become adults within 2 or 3 weeks after the culture is started. If there are large numbers of copepodids in a tray instead of adults, the food supply has probably been depleted and needs to be replenished by pouring more of the protozoan mixture into the tray so the copepodids can mature. Introduction of detritus with the food should be kept to a minimum because it can be difficult to separate copepods from detritus at the time of harvest. Copepods can be harvested by pouring the contents of a tray through a Nitex strainer (200 μ mesh) or other plastic netting[7] and rinsing the copepods from the strainer into 30 ml of dechlorinated water to concentrate them. A 300 μ mesh will capture adults and 200 μ mesh will retain copepodids. Each tray should yield about 2,000 adult copepods. A new batch of copepods can be kept in the production tray if they are not going to be used soon and maintained by adding more protozoan food culture. The rotifer *Philodina* sp. is also an excellent food source for adult copepods and can be cultured in the same way that the protozoans are cultivated. *Philodina* cultures are commercially available.[6]

Outdoor production: The scale of copepod production can be increased by outdoor production. For outdoor production we use inexpensive plastic wading pools that are 1 m wide × 8 cm deep. Alternatively, it is possible to construct wooden or concrete frames and line them with sheets of vinyl for outdoor cultures. To begin a copepod culture, we place 1 bucket (8 liters) of the protozoan culture mixture (*P. caudatum/Chilomonas* sp.), 1 bucket of dechlorinated tap water, 30 cm^2 of fresh lettuce, and about 50 adult copepods in each wading pool. Water a few centimeters deep is all that is needed for this culture, although it may be desirable to add more dechlorinated water for greater water depth at locations where evaporative losses are high. We cover the wading pools with screens sold in nurseries for shading plants. The screens prevent the water in the wading pools from overheating if the pools are exposed to direct sunlight. They also prevent invasion of outdoor cultures by invertebrates such as ostracods, cladocera or aquatic diptera, which can seriously reduce copepod production by depleting the food supply. Ostracods have been particularly important because they multiply so rapidly. A wading pool that is invaded by ostracods should be emptied, washed and dried before it is used to culture copepods.

As with indoor production, the new generation of copepods in an outdoor wading pool should be harvested in 3 weeks. We used the same procedure to harvest the copepods from wading pools as was used for the plastic trays. If the copepods are going to be kept in the pool for an extended period of time, it may be necessary to add more food, but it is often possible to maintain copepods in outdoor pools without adding food because there is a sufficient quantity of phytoplankton in the water to meet their nutritional needs.

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[7] Tetkon Inc., 333 South Highland Ave., Briarcliff Manor, NY 10510 or Florida Aqua Farms, 5532 Old St. Joe Road, Dade City, FL 33525.

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