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# CYCLOPOID COPEPODS

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ABSTRACT. Cyclopoid copepods have proved more effective for practical mosquito control than any other invertebrate predator of mosquito larvae. Their operational potential is enhanced by the fact that mass production is relatively easy and inexpensive. The exceptional potential of copepods for mosquito control was first realized about 25 years ago. Since then, laboratory experiments with copepods and mosquito larvae around the world have shown:

- Only the larger copepod species (body length > 1.4 mm) are of practical use for mosquito control.
- They kill mainly 1" instar mosquitoes. The most effective species have the capacity to kill more than 40 *Aedes* larvae/copepod/day.
- They generally kill fewer *Anopheles* larvae and even fewer *Culex* larvae.

Most field testing of copepods has been in *Aedes* container-breeding habitats. Field tests have shown that:

- The most effective copepod species maintain large populations in a container habitat for as long as there is water.
- They typically reduce *Aedes* production by 99-100%.
- They can cause local eradication of container-breeding *Aedes* mosquitoes if present in a high percentage of breeding sites.

Field surveys in *Anopheles*, floodwater *Aedes*, and *Culex* breeding habitats have shown that natural copepod populations can substantially reduce, or even eliminate, mosquito production. Field trials in temporary pools, marshes, and rice fields have demonstrated that introduction of the right copepod species to the right habitat at the right time can eliminate *Anopheles* or floodwater *Aedes* larvae. As a rule, copepods cannot eliminate *Culex* production by themselves, but they can reinforce and augment control by other methods. The only large-scale operational use of copepods to date has been in Vietnam, which has achieved local eradication of *Ae. aegypti* in hundreds of villages. Conditions in Vietnam are particularly favorable because:

- Many *Ae. aegypti* breeding sites are water storage containers that are conspicuous and easily treated.
- Motivation to maintain copepods in containers for *Ae. aegypti* control is strong because of the high incidence of dengue hemorrhagic fever.
- Copepod use is effectively managed by women's associations already experienced with neighborhood health services.

Copepods have the potential for local eradication of *Ae. aegypti* and *Ae. albopictus* in many other countries besides Vietnam. Professional capacity for copepod management and social institutions for community participation to help with implementation and maintenance are the main factors limiting broader use of copepods for operational mosquito control at the present time.

## *INTRODUCTION*

It has long been known that copepods prey on mosquito larvae (Daniels 1901, Lewis 1932, Hurlbut 1938, Lindberg 1949, Bonnet and Mukaida 1957). The exceptional potential of copepods for mosquito control was first recognized by Riviere and Thirel (1981), who observed in Tahiti that the number of *Ae. aegypti* and *Ae. po/ynesiensis* larvae was greatly reduced in ovitraps that contained *Mesocyclops aspericornis* accidentally introduced with creek water. Marten (1984) independently discovered the same for *M. aspericornis* with *Ae. albopictus* larvae in artificial containers in Hawaii, and Suarez et al. (1984) did the same for *Ae. aegypti* larvae in water storage tanks in Colombia.

Since then, copepods have proved particularly effective at eliminating *Aedes* production from water storage tanks and other container breeding habitats that have water for extended periods. In fact, the use of copepods in *Aedes* container habitats has been responsible for virtually all published instances of mosquito eradication in recent years (Marten 1990a, Nam et al. 1998, Kay and Nam 2005).

This chapter summarizes what has been learned during the past 25 years about the use of copepods for mosquito control. It reviews:

- basic biology relevant to their use for mosquito control;
- laboratory experiments to determine which copepod species prey effectively on which kinds of mosquito larvae;

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Fig. 1. Electron micrograph of a female *Mesocyclops*. Source: Michael Brown.

- field experiments to explore how effective copepods can be for mosquito control in different kinds of breeding habitats;
- practical procedures for operational use of copepods;
- how to get started using copepods.

## *BASIC COPEPOD BIOLOGY*

Copepods are among the most numerous multicellular animals on Earth. These tiny crustaceans thrive abundantly in most aquatic habitats: the water column and bottom sediments in lakes and oceans; subterranean waters; and small surface waterbodies such as temporary ponds, puddles, treeholes and even the water in bromeliad leaf cups (Williamson and Reid 2001). Many species are commensal or parasitic on vertebrate hosts such as fish and whales, or invertebrate hosts such as mollusks, sponges, and corals (Boxshall and Halsey 2004). Although the adults of some parasitic species can grow to several centimeters long, most copepods range from 0.5–1.5 mm in body length.

The word "copepod" derives from the Greek "cope" meaning oar and "podos" meaning foot, and refers to their paddle-like paired swimming legs. In the basic copepod body plan there are 4 pairs of two-branched swimming legs, each pair joined at the base by a plate which forces the legs to move together. This evolutionary design has been highly successful. There are well over 13,000 named species of copepods, currently arranged in 8 major groups or orders. Three orders dominate in fresh waters: calanoids, harpacticoids, and cyclopoids (Dussart and Defaye 2001). The calanoids are mainly herbivorous and the harpacticoids are mainly omnivorous. Most of the cyclopoids are predators. Cyclopoids (Fig. 1) are the only copepods that prey on mosquito larvae. During the rest of this chapter the word "copepod" will refer only to cyclopoids.

There are approximately 700 known species of freshwater cyclopoid copepods worldwide. Though all cyclopoids use grasping mouthparts to eat (Fig. 2), the smaller species tend to be plankton feeders, whereas the larger species tend to be aggressive predators, consuming protozoans, rotifers, and small aquatic animals (Fryer 1957a; Hutchinson 1967). Algae form part of the diet of many species, but cyclopoids fed on algae alone usually do not reproduce normally, and some species such as *Mesocyclops leuckarti* require a mixed diet including animal protein to form eggs (Wyngaard and Chinnappa 1982, Hopp et al. 1997). Fryer (1957b) described the structure and functioning of the mouthparts of *Macrocyclops albidus*, observing that it uses its

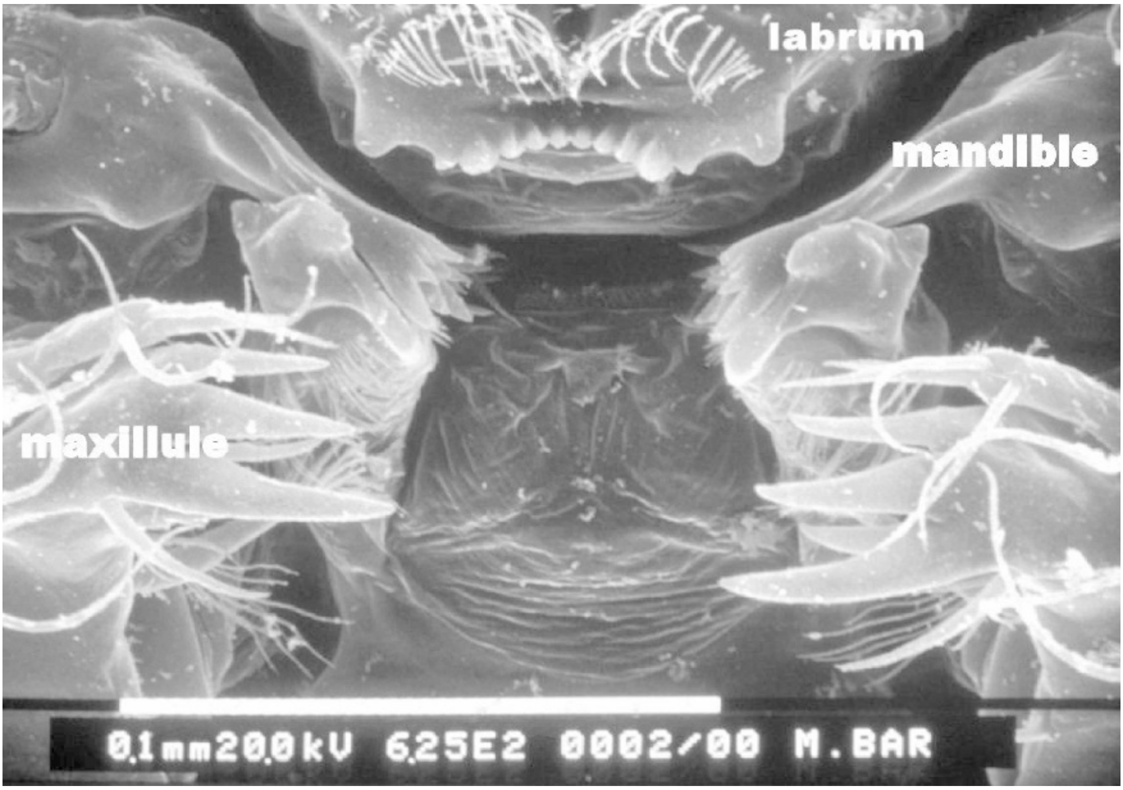

Fig. 2. Electron micrograph of *Mesocyclops* mouth parts. Source: Michael Brown.

mandibles to tear food into manageable pieces that are crammed into the esophagus without being chewed further.

Copepods have a single eye spot that senses illumination intensity (Fig. 3); thus the name "cyclops." They are active hunters, detecting their prey primarily by means of mechanoreceptors. Copepods usually swim in hops alternating with a passive sink mode, about 1 hop/sec. A hop begins with a stroke of the antennules, followed by posterior strokes of the swimming legs. If a copepod needs to escape rapidly, it can move 5 mm/sec by quickly flexing its urosome (Williamson 1986).

Although they prefer smaller prey (Brandl and Fernando 1975, Roche 1990), copepods will readily consume animals up to about twice their size. When a prey animal passes within about 1 mm of a copepod, the copepod's mechanoreceptors detect the motion in the water and it lunges at the animal. If the prey is not too large, the copepod grabs it with 3 pairs of grasping mouthparts and bites into it with its strong mandibles (Fig. 4). It will usually finish consuming a mosquito larva within a few minutes. If the prey is too large, the escape response comes into play and the copepod appears to bounce off the animal after lunging at it.

Only the larger species of copepods prey on mosquito larvae. Like larvivorous fish, these copepods are particularly effective predators for biological control because they have a broad diet that allows them to maintain large populations almost anywhere they are present – and they do so independent of the quantity of mosquito larvae as food. Though they only prey on 1st instar, and sometimes 2nd instar mosquitoes, the copepods are usually so numerous that few larvae survive to grow too large to be eaten.

The ecological versatility of copepods and their small size help them to thrive in small surface water habitats and many container habitats (e.g., rainfed tires and bromeliads) that are not suitable for fish. Copepods can kill large numbers of mosquito larvae in thick aquatic vegetation, where larvae can hide from fish (Lindberg 1949, Laird 1988). Although some kind of copepod is abundant almost everywhere there is fresh water, many sites have only species that are too small to prey on mosquito larvae. Nonetheless, the large species are common and substantially reduce larval survival, or even eliminate mosquito production completely, wherever they occur.

Copepods are sometimes found naturally in artificial containers. For example:

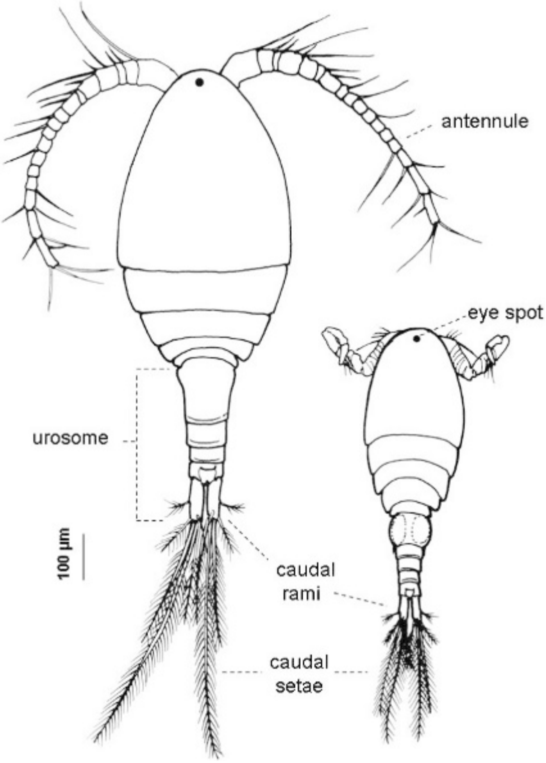

Fig. 3. Key copepod body parts. Source: Janet Reid.

- They get into discarded tires in low-lying areas, if surface water with natural copepod populations floods the tires from time to time (Marten 1989);
- Copepods can be introduced unintentionally to tanks or other containers used to store well water if there are natural copepod populations in the wells (Nam and Kay 1997a).

However, aside from these special situations it is unusual to see large copepods in artificial containers unless people put them there for mosquito control. The use of copepods for mosquito control is a matter of putting the right species of copepod into artificial containers or surface-water sites that do not already have a natural population.

Copepods reproduce sexually. After they hatch from the eggs, which are usually carried by the female in paired sacs, copepods pass through 6 nauplius stages (Fig. 5) and 5 copepodid stages, molting after each one until reaching the adult stage which does not molt. Depending on the species and environmental factors, especially temperature and food supply, copepods may mature from egg to adult within a few days to a few weeks (Wyngaard and Chinnappa 1982). In many populations, the males mature earlier than the females, ready to inseminate virgin females just after they molt to the adult stage. The male

Fig. 4. Female *Mesocyclops aspericornis* after seizing an *Ae. aegypti* larva. Source: Marco Suárez.

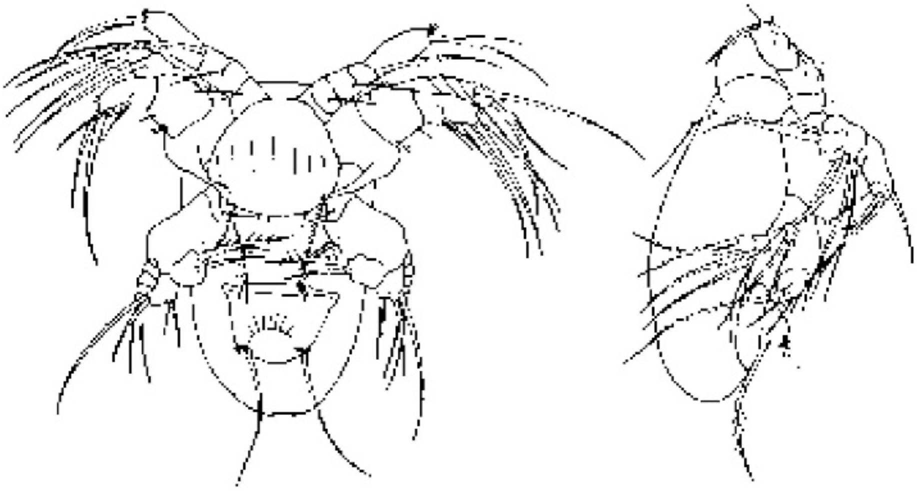

Fig. 5. Copepod nauplius (dorsal view and side view). Source: Marten et al. (1997).

attaches a pair of bean-shaped spermatophores to the female's genital opening, and the female stores the sperm to fertilize a new batch of eggs every 3–6 days for the rest of her life. The life span under optimum culture conditions is about 1–2 months (Hopp et al. 1997). Females often predominate in mature populations. Laboratory cultures can be started with females alone, and it is equally sufficient to introduce only females to mosquito breeding habitats for control purposes. The females are usually already inseminated and will quickly generate a large population if they have the food they need to produce eggs.

The capacity of copepods to enter a resting stage helps them survive in small waterbodies that dry up periodically (Williams-Howze 1997). Dormancy may range from simple quiescence, in which a copepod responds to an immediate stimulus such as temporary drying, to a true diapause in which the copepod reacts to environmental cues by slowing its metabolism and interrupting its development for long periods of time. True diapause usually occurs in particular developmental stages, pre-adults or adults, according to the species, and is widespread in freshwater cyclopoid copepods. Environmental cues include photoperiod, temperature, poor food conditions, drying of temporary pools, or a combination of these. Diapausing copepods can survive for months in the soil or sediment of temporary-water sites with no free water present (Frisch 2002).

Different species have different abilities to tolerate desiccation. Frisch and Santer (2004) observed that when 2 species of *Cyclops* were kept in humid conditions in the laboratory, diapausing copepodids of one species survived much longer than diapausing copepodids of the other species. *Acanthocyclops* and *Diacyclops*, which are highly adapted to life in temporary pools, enter diapause as a pool dries out. They can survive in dry soil for a year or more, and it is not unusual for the pools to have hundreds or thousands of active *Acanthocyclops* or *Diacyclops* as soon as there is water (Marten et al. 1994a). *Macrocyclops* and *Mesocyclops* are not so resistant to desiccation. Zhen et al. (1994) observed the survival of copepodids and adults of 4 tropical *Mesocyclops* species – *M. aspericornis*, *M. australiensis*, *M. darwini*, and *M. woutersi* (called *M. guangxiensis* at that time) – as sediment dried in experimental containers. The range of water content was comparable to that of sediments in a nearby ephemeral pond. The copepods survived in sediment with no free water as long as the water content exceeded 15%. Both copepodids and adults were swimming about soon after the containers were re-flooded with water, copepodids surviving more consistently than adult copepods. No *Mesocyclops* survived in sediments with water content less than about 15%.

There is evidence that different populations of the same copepod species may have biological differences that are important for how they function in mosquito control (Marten 1990c). For example, one strain of *Diacyclops navus* preyed on *Ae. albopictus* larvae in the laboratory, whereas another strain did not under the same experimental conditions. One strain of *Macrocyclops albidus* was better than another strain at surviving drying in tires.

## *COPEPODS AND CONTAINER-BREEDING* AEDES

### LABORATORY EXPERIMENTS

There have been numerous laboratory experiments around the world to see which species of copepods kill what kinds of mosquito larvae (Table 1). Forty-eight copepod species belonging to 15 genera have been assessed, several of them in more than one geographical region. Most of the experiments have been with container-breeding *Aedes*. The typical procedure is to put a given number of mosquito larvae in a small container with one or more copepods and count how many are killed during 24 h.

Copepod size (Table 2) is the most important factor explaining what happens in the experiments. Copepods less than 1 mm in length (e.g., *Microcyclops*, *Tropocyclops*, *Paracyclops*, and some species of *Thermocyclops*) are not likely to prey on even newly hatched mosquito larvae. Copepods around a millimeter in length (e.g., *Eucyclops*, *Ectocyclops*, most *Thermocyclops*, and some species of *Mesocyclops*) may sometimes attack 1st instar larvae but kill them only occasionally. These species are of no practical significance for mosquito control. Larger copepods such as some species of *Diacyclops* and *Acanthocyclops* kill a substantial number of larvae in the laboratory, typically 10–30 larvae/day in a small container at room temperature with an excess of larvae. The largest species (particularly *Macrocyclops*, *Megacyclops*, and *Mesocyclops* >1.4 mm body length) kill the most *Aedes* larvae, typically >40 larvae/day. The only exception to this rule of size is *Homocyclops ater*, largest of all the freshwater copepods at up to 4 mm long, which did not kill mosquito larvae in laboratory trials (Marten 1989). Some copepod species are more effective predators than other species about the same size. Species that kill the most larvae (e.g., *Mesocyclops longisetus* and *M. aspericornis*) have especially large and strong mandibles compared to their body size (Suárez-Morales et al. 2003).

Many copepod species, including some likely candidates for mosquito control, remain to be tested. In the tropics, the larger species of *Mesocyclops* have shown the best potential for control; but only 17 of the 71 presently recognized species of this genus (Ueda and Reid 2003, Hołyńska 2006) have been assessed to date. Although *Macrocyclops albidus* is an effective predator, its widely distributed congener *M. fuscus* has not been examined.

The number of *Aedes* larvae that the larger species of copepods kill is not limited by the quantity they can ingest. If there are more larvae in a laboratory container than they can eat, the copepods commonly attack one larva after another, eating only a part of each. The result is a large number of mangled and partially consumed larvae.

Copepods kill slightly fewer larvae in larger containers. A species that kills 40–50 larvae/day in a small container will kill 30–40 larvae/day in a 200-liter drum (Marten et al. 1994b). Copepods may also kill fewer larvae when alternative food (e.g., protozoa) is exceptionally abundant, but the effect of alternative food is not great enough to impact their performance for practical mosquito control (Marten 1989, 1990b).

## FIELD EXPERIMENTS

Copepods have been field-tested in a variety of container habitats around the world (Table 3). The habitats have included water storage containers such as cisterns, tanks, 200-liter drums, and large ceramic jars; also wells, bromeliads, flower vases, and containers that collect rainwater such as tires and buckets. The studies have documented the size of copepod populations that developed after introduction to the different habitats, how long the populations survived, and their impact on the survival of mosquito larvae. Most of the studies have not been on a scale that impacted the local mosquito population.

In general, copepod species that kill more larvae in the laboratory also kill more larvae in the field. *Diacyclops* and *Acanthocyclops* in New Orleans, which kill fewer mosquito larvae in the laboratory than larger copepods, are not effective enough in the field for practical mosquito control (Marten 1990b, 1990c). It is typical for *Diacyclops* to kill about 83% of the *Aedes* larvae in a tire. If the tire is crowded with larvae, the mortality due to *Diacyclops* merely thins the population, leaving a substantial number of larvae to complete their development to the adult stage. As a consequence, *Diacyclops* reduces *Aedes* production by only 20% compared to the production from control tires without copepods. *Acanthocyclops* (which has an adult body length of 1.2–1.3 mm in New Orleans) kills about 90% of the larvae, reducing the production of adult mosquitoes by 50%. This is not effective enough for practical mosquito control.

Larger copepods, including many species of *Mesocyclops*, typically kill 95–100% of the *Aedes* larvae in a container. The most effective species (e.g., *Mesocyclops longisetus*, *Mesocyclops aspericornis*, *Mesocyclops woutersi*, and *Macrocyclops albidus*) usually reduce larval survival by 99–100%. Because the larvae are not merely thinned but substantially reduced, the production of adult mosquitoes is reduced correspondingly.

One limitation of some copepod species is their tendency for unrestrained population growth in container habitats. This can lead to depletion of the food supply, stunting, and copepods that are too small to prey on mosquito larvae. It is not unusual for a single tire to contain a thousand half-sized *Diacyclops* or *Acanthocyclops*, none of which are able to prey on mosquito larvae (Marten 1990b). The most effective copepod species are not only large but also do not experience overpopulation and stunting, apparently because the adults cannibalize juveniles of their own species whenever their population starts to reduce the food supply in the container.

Is it better to introduce more than one species of copepod to a container? Mixtures of *Mesocyclops woutersi*, *Mesocyclops aspericornis*, and *Mesocyclops thermocyclopoides* have been used with excellent results in Vietnam (Nam et al. 1998). However, when mixtures of *Mesocyclops longisetus* and *Macrocyclops albidus* were introduced to tires in Louisiana, one species usually took over within a month or two (Marten 1990b). The same thing happened when mixtures of *Mesocyclops longisetus*, *Mesocyclops thermocyclopoides*, *Mesocyclops venezolanus*, and *Macrocyclops albidus* were introduced to tires and flower vases in Honduras (Marten et al. 1994b). Because the outcome was poorer if a weaker species took over, the best results were obtained by introducing only the best species (*M. longisetus* in Louisiana and Honduras).

The key factors determining which of the larger copepod species are most effective for mosquito control in a particular container habitat are:

- how long the population lasts in that kind of container;
- the number of copepods in the container.

The performance of different copepod species in different habitats can vary widely in these respects.

Copepods usually survive for as long as there is water in a container. *Mesocyclops* and *Macrocyclops* can survive in damp soil or litter, but they will not survive for long in containers (e.g., backyard buckets or discarded plastic food containers) if the container dries out or the water is poured out. Copepods can survive in bromeliads but will be lost if the bromeliads dry out. Copepods will last for years in discarded tires that get enough rain to stay wet, particularly if the tires contain leaves to retain moisture during dry periods. However, they will not survive in tires exposed to full sun with nothing inside to retain moisture. Even a few weeks without rain can dry out the tires, killing the copepods.

The number of copepods in a container depends on the food supply. Most containers that have enough natural food to support mosquito production also have enough food to support a large copepod population. If tires, cisterns, or other containers have fallen leaves or other decomposing plant material, the copepod population is large, typically thousands in a water storage tank, about a thousand in a 200-liter drum, and one or two hundred in a discarded tire. Copepod impact on larval survival is greatest under these conditions. A copepod population is much smaller in frequently cleaned tanks or other containers with little food, where their impact on larval survival may not be sufficient for mosquito control. Copepods may fail to establish large numbers and eventually die out in containers (e.g., flower vases, tires, or cement tanks) if the container is so clean that it provides little food (Jennings et al. 1993, Marten et al. 1994b). If the food supply in a container is poor, the best strategy is to add a small quantity of leaves or grain, and possibly seed the container with protozoa, to stimulate food production for the copepods (Marten 1990c, Marten et al. 1992, Dieng et al. 2003a, Kosiyachinda et al. 2003). The same food could increase the container's carrying capacity for *Aedes* larvae, but the larvae will not survive if copepods are numerous.

Water storage containers such as cisterns, cement tanks, and 200-liter drums are generally a secure habitat for copepods, but the copepods will be lost if all the water is dumped out to clean the container or if the water goes down the drain. To keep the copepods it is necessary to rescue them with a net before cleaning, holding them in a jar of water for return to the container after cleaning is finished.

In additions to hazards from cleaning, copepods in water storage containers can be lost bit-by-bit as water is removed for use. Some copepod species are more vulnerable than others. *Mesocyclops thermocyclopoides* and *Mesocyclops venezolanus* did not last long in water-storage containers in Honduras, because they swam continuously in the water column and were removed with the water (Marten et al. 1994b). Their reproductive rate was not sufficient to replace the losses, so the population gradually declined and ultimately disappeared. Fortunately, the copepod species that are most effective for mosquito control (e.g., *Macrocyclops albidus*, *Mesocyclops longisetus*, *Mesocyclops aspericornis* and *Mesocyclops woutersi*) are resistant to removal with the water because they cling to the sides of the container, rest on the bottom, or swim very close to the bottom where they are unlikely to be removed.

Temperature can limit copepod survival. *Macrocyclops albidus* is a temperate species with a global distribution. It is limited to habitats in the tropics that do not experience high temperatures. *M. albidus* did not consistently survive for long periods when introduced to 200-liter drums in Honduras, because the water sometimes became too hot in drums exposed to the afternoon sun (Marten et al. 1994b). In contrast, the genus *Mesocyclops* consists primarily of tropical species that can survive water temperatures up to 42–43°C (Table 4), though they are killed by even brief exposure to temperatures in the range of 1–8°C (depending on the species). These *Mesocyclops* have no trouble in water exposed to the sun, but they can be vulnerable to cold temperatures at the northern edge of their geographic range. For example, *Mesocyclops longisetus* is a neotropical species naturally found

Table 1. Laboratory experiments for copepod predation on mosquito larvae.

| Locality<br>Mosquito species | Copepod species tested | Reference |
|---|---|---|
| | **North America** | |
| Alabama | | |
| *An. quadrimaculatus* | *Microcyclops varicans* * | Hurlbut (1938) |
| California | | |
| *Cx. quinquefasciatus* | *Mesocyclops aspericornis* [as *M. leuckarti pilosa*] (doubtful record) | Mian et al. (1986) |
| Louisiana | *Diacyclops navus* [as *Thermocyclops dybowskii* (Landé)] | Nasci et al. (1987) |
| Species not mentioned | *Macrocyclops albidus* | |
| Louisiana | | |
| *Ae. albopictus* | *Acanthocyclops vernalis* | Marten (1989, 1990b) |
| | *Diacyclops navus* | |
| | *Macrocyclops albidus* | |
| | *Mesocyclops edax* | |
| | *Mesocyclops longisetus* | |
| | *Mesocyclops pehpeiensis* [as *Mesocyclops* sp. *leuckarti* group] | |
| | *Apocyclops panamensis* * | |
| | *Ectocyclops rubescens* * | |
| | *Eucyclops agilis* * | |
| | *Eucyclops elegans* [as *Eucyclops speratus* (Lilljeborg)] * | |
| | *Homocyclops ater* * | |
| | *Megacyclops latipes* | |
| | *Metacyclops cushae* [as *Metacyclops denticulatus* Dussart and Frutos] * | |
| | *Microcyclops varicans* * | |
| | *Orthocyclops modestus* * | |
| | *Paracyclops chiltoni* [as *Paracyclops fimbriatus* (Fischer)] * | |
| | *Paracyclops poppei* * | |
| | *Thermocyclops inversus* * | |
| | *Tropocyclops prasinus* * | |
| Louisiana | | |
| *Ae. aegypti* | *Diacyclops navus* | Reid et al. (1989) |
| Connecticut | | |
| *Ae. canadensis* | *Acanthocyclops vernalis* | Andreadis and Gere (1992) |
| *Ae. stimulans* | *Diacyclops thomasi* [as *Diacyclops bicuspidatus thomasi*]* | |
| Louisiana | | |
| *Ae. albopictus* | *Macrocyclops albidus* | Marten et al. (1994a) |
| *Ae. sollicitans* | *Mesocyclops longisetus* | |
| *An quadrimaculatus* | | |
| *Cx. quinquefasciatus* | | |
| *Cx. restuans* | | |
| *Cx. salinarius* | | |
| Nuevo León, Mexico | | |
| *Ae. aegypti* | *Mesocyclops longisetus* | Pérez-Serna et al. (1996) |
| *Cx. pipiens* | *Macrocyclops albidus* | |
| Louisiana | | |
| *Cx. quinquefasciatus* | *Acanthocyclops vernalis* | Marten et al. (2000b) |
| | *Macrocyclops albidus* | |
| | *Megacyclops latipes* | |
| Louisiana | | |
| *An. quadrimaculatus* | *Acanthocyclops vernalis* | Marten et al. (2000a) |
| | *Macrocyclops albidus* | |
| | *Mesocyclops longisetus* | |
| | *Mesocyclops pehpeiensis* | |
| | *Megacyclops latipes* | |
| Florida | | |
| *Ae. aegypti* | *Macrocyclops albidus* | Rey et al. (2004) |
| *Ae. albopictus* | | |
| Florida | | |
| *Ae. albopictus* | *Mesocyclops longisetus* | Soumare et al. (2004) |
| *Cx. quinquefasciatus* | | |

Table 1. Continued

| Locality<br>Mosquito species | Copepod species tested | Reference |
|---|---|---|
| | **Central America, Caribbean** | |
| Honduras | | |
| *Ae. aegypti* | *Macrocyclops albidus*<br>*Mesocyclops longisetus*<br>*Mesocyclops thermocyclopoides*<br>*Mesocyclops venezolanus*<br>*Acanthocyclops smithae* [as *Acanthocyclops* sp. *vernalis* group]*<br>*Ectocyclops rubescens**<br>*Eucyclops agilis**<br>*Mesocyclops pescei**<br>*Mesocyclops reidae** | Marten et al. (1994b) |
| Trinidad | | |
| *Ae. aegypti* | *Macrocyclops albidus*<br>*Mesocyclops aspericornis*<br>*Mesocyclops longisetus* | Rawlins et al. (1997) |
| Costa Rica | | |
| *Ae. aegypti* | *Mesocyclops thermocyclopoides* | Schaper et al. (1998) |
| Cuba | | |
| *Ae. aegypti* | *Macrocyclops albidus* | Menéndez-Díaz et al. (2004) |
| | **South America** | |
| Colombia | | |
| *Ae. aegypti* | *Mesocyclops aspericornis* | Suárez et al. (1984) |
| Colombia | | |
| *An. albimanus*<br>*Culex* sp. | *Apocyclops panamensis* *<br>*Diacyclops hispidus*<br>*Ectocyclops rubescens* *<br>*Eucyclops agilis* *<br>*Eucyclops bondi* *<br>*Macrocyclops albidus*<br>*Mesocyclops aspericornis*<br>*Mesocyclops longisetus*<br>*Mesocyclops venezolanus*<br>*Microcyclops anceps* *<br>*Thermocyclops decipiens* *<br>*Thermocyclops tenuis** | Marten et al. (1989) |
| Brazil | | |
| *Ae. aegypti*<br>*An. farauti*<br>*Cx. quinquefasciatus* | *Mesocyclops aspericornis*<br>*Mesocyclops longisetus* | Kay et al. (1992b) |
| Brazil | | |
| *Ae. albopictus*<br>*Ae. aegypti* | *Macrocyclops albidus*<br>*Mesocyclops longisetus*<br>*Eucyclops ensifer**<br>*Eucyclops serrulatus**<br>*Metacyclops mendocinus** | Santos and Andrade (1997) |
| Argentina | | |
| *Ae. aegypti*<br>*Cx. pipiens* | *Mesocyclops annulatus* | Micieli et al. (2002) |
| Uruguay | | |
| *Cx. pipiens* | *Macrocyclops albidus*<br>*Mesocyclops longisetus*<br>*Acanthocyclops robustus**<br>*Eucyclops neumanni**<br>*Metacyclops grandis**<br>*Metacyclops mendocinus** | Calliari et al. (2003) |
| | **Asia, Middle East** | |
| Iran | | |
| *An. superpictus* | *Megacyclops viridis* | Lindberg (1949) |

Table 1. Continued

| Locality<br>Mosquito species | Copepod species tested | Reference |
|---|---|---|
| Singapore | | |
| Anopheline and culicid larvae | *Mesocyclops aspericornis*<br>*Mesocyclops thermocyclopoides* | Laird (1988) |
| Israel | | |
| *Ae. aegypti*<br>*Cs. longiareolata*<br>*Cx. pipiens* | *Megacyclops viridis* [as *Acanthocyclops viridis*] | Blaustein and Margalit (1994) |
| India | | |
| *Cx. quinquefasciatus* | *Mesocyclops leuckarti* sensu lato (identification uncertain) | Bapna and Renapurkar (1994) |
| Indonesia | | |
| *Ae. aegypti*<br>*An. aconitus*<br>*Cx. quinquefasciatus*<br>*An. stephensi* | *Mesocyclops aspericornis* | Yuniarti et al. (1995)<br>Widyastuti and Yuniarti (1997) |
| *Ae. aegypti*<br>*Cx. quinquefasciatus* | *Mesocyclops thermocyclopoides* | Mittal et al. (1997)<br>India |
| Vietnam | | |
| *Ae. aegypti* | *Mesocyclops affinis*<br>*Mesocyclops aspericornis*<br>*Mesocyclops ogunnus*<br>*Mesocyclops pehpeiensis*<br>*Mesocyclops thermocyclopoides* | Nam et al. (1999) |
| Japan | | |
| *Ae. albopictus*<br>*Cx. tritaeniorhynchus*<br>*An. minimus* | *Macrocyclops distinctus*<br>*Megacyclops viridis*<br>*Mesocyclops pehpeiensis* | Dieng et al. (2003a) |
| India | | |
| *An. stephensi*<br>*Cx. quinquefasciatus* | *Mesocyclops thermocyclopoides* | Kumar and Ramakrishna Rao (2003) |
| Thailand | | |
| Water-storage containers<br>*Ae. aegypti* | *Mesocyclops aspericornis* | Kosiyachinda et al. (2003) |
| Thailand | | |
| *Ae. aegypti* | *Mesocyclops thermocyclopoides* | Chansang et al. (2004) |
| Philippines | | |
| *Ae. aegypti* | *Mesocyclops aspericornis*<br>*Mesocyclops ogunnus** | Panogadia-Reyes et al. (2004) |
| | **Australia** | |
| Queensland | | |
| *Ae. aegypti*<br>*An. farauti*<br>*Cx. quinquefasciatus* | *Mesocyclops acanthoramus* [as *Mesocyclops* mb3]<br>*Mesocyclops affinis* [as *Mesocyclops* mb1]<br>*Mesocyclops aspericornis*<br>*Mesocyclops australiensis*<br>*Mesocyclops darwini*<br>*Mesocyclops notius**<br>*Mesocyclops woutersi* [probably as *Mesocyclops* mb2] | Brown et al. (1991a, 1991b) |
| Queensland | | |
| *Ae. aegypti* | *Mesocyclops aspericornis* | Russell et al. (1996) |
| | **Oceania** | |
| Hawaii | | |
| *Tx. brevipalpis* | *Mesocyclops aspericornis* [as *Mesocyclops obsoletus* (Koch)] | Bonnet and Mukaida (1957) |
| Tahiti | | |
| *Ae. aegypti*<br>*Ae. polynesiensis*<br>*Cx. quinquefasciatus*<br>*Tx. amboinensis* | *Mesocyclops aspericornis* [as *Mesocyclops leuckarti f. pilosa* Kiefer] | Rivière and Thirel (1981) |
| Hawaii | | |
| *Ae. albopictus* | *Mesocyclops aspericornis* [as *M. leuckarti pilosa*] | Marten (1984) |
| | **Africa** | |
| Malawi | | |
| Anopheline larvae | "*Cyclops*" (species undetermined) | Daniels (1901) |

Table 1. Continued

| Locality<br>Mosquito species | Copepod species tested | Reference |
|---|---|---|
| | **Europe** | |
| U.K. | | |
| *An. bifurcatus* (= *An. claviger* Meigen) | "*Cyclops*" (species undetermined) | Lewis (1932) |

only in deep water (e.g., canals) in Louisiana, apparently because it is killed in shallower water during exceptionally cold periods in winter. In contrast, *Macrocyclops albidus* can survive for years in water at 0°C, though it is killed if the water freezes solid.

Copepods tolerate a pH range of 5–9 (Marten [NOMCB] August 1993 p. 6, Jennings et al. 1994). *Mesocyclops aspericornis* and *M. darwini* were observed to tolerate salinities up to approximately 1000 ppm (Jennings et al. 1994). Copepods are sensitive to heavy metals such as copper, chromium, nickel, and zinc (Wong and Pak 2004). The toxic substance of greatest practical significance is chlorine in tap water. The U.S. Environmental Protection Agency standard of 0.2 ppm chlorine for tap water is precariously close to the tolerance of copepods, which have an LD$_{50}$ of 0.5–1.0 ppm for chlorine, depending on the copepod species (Brown et al. 1994b). However, the chlorine in tap water is substantially less than 0.2 ppm in many localities. In some parts of developing countries, there may be no chlorine at all in the water.

Table 2. Body lengths of adult females of cyclopoid copepods. Lengths are given in approximate descending order and do not include the antennules or caudal setae.[1,2]

| Species | Length (mm) | Species | Length (mm) |
|---|---|---|---|
| Predators of mosquito larvae | | Not predators of mosquito larvae | |
| *Megacyclops latipes* (Lowndes) | 1.8–2.5 | *Homocyclops ater* (Herrick) | 1.8–4.0 |
| *Macrocyclops albidus* (Jurine) | 1.7–2.5 | *Acanthocyclops robustus* (G. O. Sars) | 1.0–2.0 |
| *Macrocyclops fuscus* (Jurine) | 1.8–2.2 | *Metacyclops grandis* (Kiefer) | 1.5–1.6 |
| *Macrocyclops distinctus* (Richard) | 1.8–2.2 | *Eucyclops elegans* (Herrick) | 1.0–1.6 |
| *Megacyclops viridis* (Jurine) | 1.2–2.1 | *Eucyclops neumanni* (Pesta) | 1.0–1.5 |
| *Mesocyclops annulatus* (Wierzejski) | 1.3–2.0 | *Eucyclops agilis* (Koch) | 0.8–1.4 |
| *Acanthocyclops vernalis* (Fischer) | 1.2–2.0 | *Eucyclops serrulatus* (Fischer) | 0.8–1.4 |
| *Mesocyclops longisetus* (Thiébaud) | 1.2–2.0 | *Diacyclops thomasi* (S.A. Forbes) | 0.8–1.4 |
| *Mesocyclops longisetus curvatus* Dussart | 1.2–2.0 | *Microcyclops anceps* (Richard) | 0.7–1.4 |
| *Mesocyclops pehpeiensis* Hu | 1.1–1.7 | *Orthocyclops modestus* (Herrick) | 0.8–1.3 |
| *Mesocyclops aspericornis* (Daday) | 1.1–1.6 | *Ectocyclops rubescens* Brady | 0.9–1.2 |
| *Mesocyclops affinis* Van de Velde | 0.9–1.6 | *Eucyclops ensifer* Kiefer | 0.9–1.2 |
| *Mesocyclops edax* (S. A. Forbes) | 0.8–1.6 | *Mesocyclops reidae* Petkovski | 0.8–1.2 |
| *Mesocyclops ogunnus* Onabamiro | 1.0–1.3 | *Metacyclops mendocinus* (Wierzejski) | 0.8–1.2 |
| *Mesocyclops woutersi* Van de Velde | 1.0–1.3 | *Acanthocyclops smithae* Reid and Suárez-Morales | 0.9–1.1 |
| *Mesocyclops venezolanus* Dussart | 1.0–1.2 | *Thermocyclops tenuis* (Marsh) | 0.8–1.1 |
| *Mesocyclops acanthoramus* Hołyńska and Brown | 1.0–1.2 | *Thermocyclops decipiens* (Kiefer) | 0.7–1.0 |
| *Diacyclops navus* (Herrick) | 0.8–1.3 | *Diacyclops hispidus* Reid | 0.9–1.0 |
| *Mesocyclops darwini* Dussart and Fernando | 0.9–1.3 | *Microcyclops varicans* (G. O. Sars) | 0.5–1.0 |
| *Mesocyclops thermocyclopoides* Harada | 0.8–1.2 | *Mesocyclops notius* Kiefer | 0.8–0.9 |
| *Mesocyclops australiensis* (G. O. Sars) | 0.8–1.1 | *Paracyclops chiltoni* (Thomson) | 0.7–0.9 |
| | | *Paracyclops poppei* (Rehberg) | 0.7–0.9 |
| | | *Ectocyclops rubescens* Brady | 0.7–0.9 |
| | | *Tropocyclops prasinus* (Fischer) | 0.5–0.9 |
| | | *Eucyclops bondi* Kiefer | 0.7–0.8 |
| | | *Mesocyclops pescei* Petkovski | 0.6–0.8 |
| | | *Metacyclops cushae* Reid | 0.6–0.8 |
| | | *Apocyclops panamensis* (Marsh) | 0.6–0.7 |
| | | *Thermocyclops inversus* Kiefer | 0.6–0.7 |
| | | *Microcyclops alius* (Kiefer) | 0.5–0.7 |

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[1] Sources: Einsle (1993), Holynska and Brown (2003), Reid (1985, 1991), Reid and Suárez-Morales (1999), Ueda and Reid (2003), and Yeatman (1959).

[2] The range of body lengths for some species in the table is large because they are species groups containing species of different sizes. The body length of individual species can vary with nutritional state, time of year, or geographical region.

Table 3. Field studies of copepod predation on mosquito larvae.

| Location<br>Microhabitat<br>Mosquito species | Copepod species | Reference |
|---|---|---|
| **North America** | | |
| Louisiana<br>Tires, buckets<br>*Ae. aegypti*<br>*Ae. albopictus*<br>*Ae. triseriatus* | *Acanthocyclops vernalis*<br>*Diacyclops navus*<br>*Macrocyclops albidus*[a]<br>*Mesocyclops edax*<br>*Mesocyclops longisetus*[a]<br>*Mesocyclops pehpeiensis* [as *Mesocyclops leuckarti* species-group or *Mesocyclops ruttneri*] | Marten (1989, 1990a, 1990b)<br>Marten et al. (1994a) |
| Florida<br>Tire piles<br>*Ae. albopictus* | *Acanthocyclops vernalis*<br>*Mesocyclops longisetus* | Schreiber et al. (1993) |
| Nuevo León, Mexico<br>Drums<br>*Ae. aegypti* | *Mesocyclops longisetus* | Quiroz-Martínez et al. (1993) |
| Louisiana<br>Temporary pools, Spartina marsh,<br>*Oc. sollicitans*<br>*Ae. vexans*<br>*Cx. salinarius*<br>*An. crucians* | *Acanthocyclops vernalis*<br>*Macrocyclops albidus*<br>*Mesocyclops longisetus* | Marten et al. (1994a) |
| Honduras<br>Tires, cement tanks, drums, flower vases<br>*Ae. aegypti* | *Macrocyclops albidus*<br>*Mesocyclops longisetus*<br>*Mesocyclops thermocyclopoides*<br>*Mesocyclops venezolanus* | Marten et al. (1994b) |
| Florida<br>Tires<br>*Ae. albopictus*<br>*Cx. quinquefasciatus*<br>*Cx. salinarius*<br>*Cx. territans*<br>*Tx. rutilus rutilus* | *Mesocyclops longisetus*[a] | Tietze et al. (1994) |
| Florida<br>Tires<br>*Ae. albopictus*<br>*Cx. quinquefasciatus*<br>*Cx. salinarius*<br>*Oc. triseriatus*<br>*Cx. restuans*<br>*Or. signifera* | *Mesocyclops longisetus* | Schreiber et al. (1996) |
| Nuevo León, Mexico<br>Drums, tires, cemetery flower vases<br>*Ae. aegypti* | *Mesocyclops longisetus* | Gorrochoteguei-Escalante et al. (1998) |
| Yucatán, Mexico<br>Tires<br>*Ae. aegypti* | *Mesocyclops longisetus* | Manrique-Saide et al. (1998) |
| Louisiana<br>Rice fields<br>*An. quadrimaculatus* | *Acanthocyclops vernalis*<br>*Macrocyclops albidus*<br>*Mesocyclops edax*<br>*Mesocyclops longisetus*<br>*Mesocyclops pehpeiensis* [as *Mesocyclops ruttneri*] | Marten et al. (2000a) |
| Louisiana<br>Roadside ditches<br>*Cx. quinquefasciatus* | *Macrocyclops albidus* | Marten et al. (2000b) |

Table 3. Continued

| Location<br>Microhabitat<br>Mosquito species | Copepod species | Reference |
|---|---|---|
| Florida<br>Tires<br>*Ae. aegypti*<br>*Ae. albopictus* | *Macrocyclops albidus* | Rey et al. (2004) |
| | **Central America, Caribbean** | |
| Honduras<br>Drums, tires, artificial containers<br>*Ae. aegypti* | *Macrocyclops albidus*[a]<br>*Mesocyclops longisetus* var. *curvatus*[a]<br>*Mesocyclops thermocyclopoides*[a]<br>*Mesocyclops venezolanus*[a] | Marten et al. (1992, 1994a, 1994b) |
| Puerto Rico, Anguilla<br>Drums, tires | *Mesocyclops aspericornis* | Suárez (1992) |
| Costa Rica<br>Artificial containers, bromeliads<br>*Ae. aegypti* | *Mesocyclops thermocyclopoides*[a] | Schaper et al. (1998), Schaper (1999), Soto et al. (1999), Hernández-Chavarría and García (2000) |
| | **South America** | |
| Colombia<br>Surface water<br>*An. albimanus* | *Mesocyclops aspericornis*<br>*Mesocyclops longisetus*<br>*Mesocyclops venezolanus* | Marten et al. (1989, 1996)[b] |
| Brazil<br>Tires<br>*Ae. albopictus* | *Mesocyclops longisetus* | Santos et al. (1996) |
| Brazil<br>Wells, water-storage containers<br>*Ae. aegypti* | *Mesocyclops longisetus* | Vasconcelos et al. (1992) |
| Venezuela<br>Marsh<br>*An. aquasalis* | *Mesocyclops longisetus*<br>*Mesocyclops meridianus* | Zoppi de Roa et al. (2002) |
| Argentina<br>Artificial containers<br>*Ae. aegypti* | *Mesocyclops annulatus* | Marti et al. (2004) |
| Colombia<br>Catch basins<br>*Ae. aegypti* | *Mesocyclops longisetus* | Suárez-Rubio and Suárez (2004) |
| | **Asia, Middle East** | |
| Lao People's Republic<br>Wells, water-storage containers<br>*Ae. aegypti*<br>*Cx. quinquefasciatus*<br>*An. maculates* | *Mesocyclops woutersi* [as *Mesocyclops guangxiensis*]<br>*Mesocyclops aspericornis* | Jennings et al. (1995) |
| Vietnam<br>Wells, water-storage containers<br>*Ae. aegypti* | *Mesocyclops pehpeiensis* [as *Mesocyclops ruttneri*][a]<br>*Mesocyclops thermocyclopoides*[a]<br>*Mesocyclops woutersi*[a]<br>*Mesocyclops aspericornis*[a] | Nam et al. (1997b, 1998, 2005)<br>Kay et al. (2002b, 2005) |
| Japan<br>Artificial containers<br>*Ae. albopictus* | *Macrocyclops distinctus*<br>*Megacyclops viridis*<br>*Mesocyclops pehpeiensis* | Dieng et al. (2002, 2003b) |

Table 3. Continued

| Location<br>Microhabitat<br>Mosquito species | Copepod species | Reference |
|---|---|---|
| Lao People's Republic | | |
| Water storage containers | *Mesocyclops aspericornis* | Tsuda et al. (2002) |
| Discarded containers | | |
| Philippines | | |
| Drums | *Mesocyclops aspericornis* | Panogadia-Reyes et al. (2004) |
| *Ae. aegypti* | *Mesocyclops ogunnus* | |
| | **Australia** | |
| Queensland | | |
| Water tanks | *Mesocyclops aspericornis* | Jennings et al. (1993, 1994) |
| *Ae. aegypti* | | |
| Queensland | | |
| Water tanks, tires | *Mesocyclops aspericornis* | Brown et al. (1992, 1994a, 1996) |
| *Ae. aegypti* | | |
| Queensland | | |
| Mine wells | *Mesocyclops aspericornis* | Russell et al. (1996) |
| *Ae. aegypti* | | |
| Queensland | | |
| Service manholes, pits | *Mesocyclops acanthoramus* [as *Mesocyclops* sp. 1] | Kay et al. (2000, 2002a) |
| *Oc. tremulus* | *Mesocyclops aspericornis* | |
| *Ae. aegypti* | *Mesocyclops darwini* | |
| | **Oceania** | |
| Tahiti | | |
| Ovitraps, tires, land-crab burrows, treeholes, drums, wells, cisterns | *Mesocyclops aspericornis* [as *Mesocyclops leuckarti pilosa*] | Rivière and Thirel (1981)<br>Rivière (1985) |
| *Ae. aegypti* | | Rivière et al. (1987a, 1987b, 1998) |
| *Ae. polynesiensis* | | |
| Hawaii | | |
| Jars | *Mesocyclops aspericornis* [as *Mesocyclops leuckarti pilosa*] | Marten (1984) |
| *Ae. albopictus* | | |
| French Polynesia | | |
| Drums, tires, cisterns, land-crab burrows | *Mesocyclops aspericornis*[a] | Lardeux (1992) |
| *Ae. aegypti* | | Lardeux et al. (1989, 1992, 2002a, 2002b) |

[a] Part of integrated control measures (e.g., reduction of breeding sites, treatment with BTI or methoprene, or addition of other larval predators).

[b] Field survey.

With regard to mosquito insecticides, copepods are completely unaffected by *Bacillus thuringiensis israelensis* (*Bti*) and tolerant of permethrin, methoprene, and pyriproxygen (Bircher and Ruber 1988, Marten et al. 1993, Wang et al. 2005). They are readily killed by temephos and malathion.

## LARGE-SCALE FIELD TRIALS

Rivière et al. (1987a, 1987b) conducted the first large-scale copepod field trials by introducing *Mesocyclops aspericornis* to crabholes in Tahiti where *Ae. polynesiensis* and *Ae. aegypti* were breeding. *Mesocyclops aspericornis* reduced larval survival by 91–99% wherever the copepods were present. Although the scale of the introductions was large enough to expect an impact on the mosquito population, there was no significant long-term effect because the copepods failed to survive when crab holes dried out.

The first demonstration that copepods can eradicate local mosquito populations was achieved in New Orleans (Marten 1990a, Weiss 1990). Tires containing *Macrocyclops albidus* were placed in woodlots that contained *Ae. albopictus*. Year-round rainfall, shade in the woodlots, and leaf litter in the tires ensured that there was always enough moisture for copepod survival. *Aedes albopictus* populations around the tire piles declined to zero over a period of 5 months and did not reappear during the following 3 years of observation.

Lardeux (1992) introduced *Mesocyclops aspericornis* to all the water storage tanks and 200-liter drums in a village in French Polynesia. *Aedes aegypti* production was suppressed in the water-storage containers where *M. aspericornis* established a population, but the copepods did not survive in enough of the containers to have a significant impact on the mosquito population.

Table 4. Minimum and maximum temperatures survived by copepods during one day of exposure in the laboratory.[1]

| Copepod species[2] | Temperature (degrees C) Minimum | Temperature (degrees C) Maximum |
|---|---|---|
| *Mesocyclops venezolanus* (H) | 8 | 42 |
| *Mesocyclops aspericornis* (PR) | 5 | 43 |
| *Mesocyclops thermocyclopoides* (H) | 4 | 42 |
| *Mesocyclops longisetus* (H) | 3 | 42 |
| *Mesocyclops pehpeiensis* (NO) | 1 | 42 |
| *Mesocyclops longisetus* (NO) | 1 | 41 |
| *Megacyclops latipes* (NO) | 0 | 39 |
| *Mesocyclops edax* (NO) | 0 | 38 |
| *Acanthocyclops vernalis* (NO) | 0 | 38 |
| *Macrocyclops albidus* (H) | 0 | 37 |
| *Macrocyclops albidus* (PR) | 0 | 37 |
| *Macrocyclops albidus* (NO) | 0 | 37 |

[1] Source: Marten et al. (1994a).

[2] Collection locations: H = Honduras, PR = Puerto Rico, NO = New Orleans.

As part of a community-based dengue control program in Honduras (Fernández et al. 1992), housewives in a small urban neighborhood maintained *Mesocyclops longisetus* in 200-liter drums (*pilas*) and flower vases (Marten et al. 1992, 1994a, 1994b). Intensive community organizing was necessary because the requisite neighborhood organization did not already exist. The housewives did an outstanding job of maintaining copepods in the vases and drums, virtually eliminating *Ae. aegypti* production from those containers. However, the copepods did not work so well in small cement tanks (capacities of several hundred liters) attached to every house to store water for laundry and other household cleaning. Detergent and bleach toxic to copepods went into tank water as women ladled water out of the tanks to wash clothes on a washboard beside the tank, and copepods were flushed down the drain when the tanks were cleaned. Juvenile turtles provided excellent mosquito control in the tanks because they were unaffected by household chemicals in the water and too large to go down the drain (Borjas et al. 1993). However, the combination of source reduction, copepods, and turtles, which was so effective in one small neighborhood, never expanded to a larger scale because intensive community organization on that scale was beyond the government's capacity.

The New Orleans Mosquito Control Board (NOMCB) has successfully eliminated *Ae. albopictus* production in thousands of tires by introducing *Mesocyclops longisetus* (Marten et al. 1994a). Treating tire piles or other large concentrations of discarded tires reduced mosquito populations in the immediate vicinity of the tires, but the impact on *Ae. albopictus* populations throughout the city has been negligible. It has not been feasible to mount the kind of integrated community-based mosquito control that would be necessary to deal with the staggering abundance and variety of breeding containers prevailing in so many of the city's residential areas.

Kay et al. (2000) surveyed service manholes and pits in northern Queensland stormdrains, where *Oc. tremulus*, *Oc. notoscriptus*, and *Ae. aegypti* breed. There was a strong negative association between the presence of *Mesocyclops* sp. (presumably *M. aspericornis* and *M. darwini*) and the presence of *Ochlerotatus* or *Aedes* larvae. Subsequent *Mesocyclops* introductions to stormdrains demonstrated how effective the copepods could be for mosquito control (Kay et al. 2002a). Fifty *Mesocyclops* sp. were introduced to a single service manhole in Townsville, Queensland. All manholes in the vicinity were monitored for 1 year before *Mesocyclops* introduction and for 3 years afterwards. *Mesocyclops* spread to manholes as far away as 2 km from the introduction site by the year after introduction, reaching 83% of the manholes in an area of 1.3 km$^2$ by the 3rd year. Once in a manhole, the copepods stayed year after year. They were not washed out of the manholes by high water flows, and they survived in damp sediment during dry periods. Over the entire monitoring period, 11% of the manhole inspections without *Mesocyclops* were positive for *Ochlerotatus* or *Aedes* larvae, which usually numbered several thousand. In contrast, only 3% of the inspections of manholes with *Mesocyclops* revealed any larvae at all. The absolute impact of copepods on *Ochlerotatus* and *Aedes* production became particularly clear when 50 *M. aspericornis* and *M. darwini* were introduced to 4 stormdrain service pits where thousands of larvae had been found. Within 4–6 months, the number of *Ochlerotatus* and *Aedes* larvae declined to zero and remained at zero during an additional year of monitoring.

Suárez-Rubio and Suárez (2004) introduced *Mesocyclops longisetus* to 200 catch basins in Colombia. The copepods established large populations in 50% of the basins, which had low numbers of *Ae. aegypti* larvae once *M. longisetus* became numerous.

## OPERATIONAL USE IN VIETNAM

The preeminent success story for operational use of copepods has come from Vietnam (Nam et al. 1997a, 1997b, 1998, 2000, 2005; Marten 2000, 2001, p. 184–196; Kay et al. 2001, 2002b, Kay and Nam 2005). In 1993, scientists at Vietnam's National Institute of Epidemiology and Hygiene introduced a mixture of *Mesocyclops woutersi*, *Mesocyclops thermocyclopoides*, and *Mesocyclops pehpeiensis* to all the wells, cement water storage tanks (average capacity 2700 liters), and ceramic jars (average capacity 27 liters) in Phanboi, a village of 400 houses in northern Vietnam. The *Ae. aegypti* population declined to 3% of its former density over a period of 12 months, but the mosquitoes did not disappear entirely. When plastic containers that collected rainwater while waiting for recycling pickup were brought together and stored so they would not collect rainwater, the mosquito population declined to zero within another 8 months. No *Ae. aegypti* have been seen in the village since then.

Since the success in Phanboi, the use of copepods in combination with appropriate source reduction has eradicated *Ae. aegypti* in villages and urban neighborhoods with a total population of approximately 400,000 people (Kay and Nam 2005). In every instance, *Ae. aegypti* disappeared or declined to very low numbers within about a year after copepod introduction. Copepods have been the decisive factor. Source reduction in Vietnam without the use of copepods has had negligible impact on *Ae. aegypti* populations.

The practical procedure for copepod use in Vietnam is straightforward. A government health worker explains their use to the local women's union, which already does other health activities such as immunization and family planning on a door-to-door basis. A small number of copepods are introduced to one of the village water storage tanks, where the copepods multiply to thousands within 1 or 2 months. Copepods are distributed from there by carrying buckets of water containing copepods around the village and ladling a small amount of the water into all appropriate containers. A key to success is training local "health collaborators" to maintain a neighborhood monitoring system, periodically checking every household to confirm that copepods are still there. If a container is missing copepods, they are easily reintroduced from one that has them. School children contribute through campaigns to collect and remove discarded containers.

It is not necessary to have copepods in every container to achieve complete eradication of local mosquito populations. Village-level *Ae aegypti* eradication in Vietnam has succeeded with copepods present in only about 90% of the water-storage tanks and even fewer of the other containers. The fundamental reason for success without complete coverage of the containers is the "egg trap effect." Treating mosquito-breeding habitats with copepods is more effective than eliminating the habitats, because copepods convert the habitats into egg sinks. The adult mosquito population is generally proportional to the carrying capacity of the larval habitat, so eliminating 90% of the breeding habitats with conventional source reduction will reduce the adult population by 90%. However, experience in Vietnam has shown that *Ae. aegypti* populations collapse when 90% of the breeding habitats are converted to egg sinks with copepods (Nam et al. 1998). This happens because mosquitoes that emerge from untreated containers waste most of their eggs on containers with copepods.

The egg trap effect would be reduced if the presence of copepods in the water repelled mosquito oviposition. It would be augmented if copepods attracted oviposition. Torres-Estrada et al. (2001) reported that *Mesocyclops longisetus* attracted oviposition by *Ae. aegypti*. Laboratory and field experiments in New Orleans have confirmed that *Macrocyclops albidus* and *Mesocyclops longisetus* definitely do not repel oviposition by *Ae. albopictus* or *Ae. aegypti*. The copepods sometimes attract *Aedes* to lay up to twice as many eggs compared to containers without copepods, but the attraction is not consistent (G.G. Marten, G. Thompson, and M. Nguyen, unpublished data).

## *PRACTICAL PROCEDURES*

The New Orleans Mosquito Control Board prepared a comprehensive manual that explains in detail practical procedures for copepod mass production and operational use (Marten et al. 1997).

## MASS PRODUCTION

Containers of any size or shape can be used for mass production of copepods. Food supply is the key to success. The first production system used *Chlorella* algae, rotifers, and *Paramecium caudatum* (Rivière et al. 1987a), but a combination of *Paramecium caudatum* and the flagellate *Chilomonas* has proved to be the most easily managed and nutritious food for most copepod species that are used for mosquito control (Suárez et al. 1992, Marten et al. 1997). *Chilomonas* provides small-sized food for the copepod nauplii, and *Paramecium* provides larger food for copepodids and adults. One significant exception to producing copepods with a diet of *Chilomonas* and *P. caudatum* is *Megacyclops viridis*, which requires rotifers (e.g., *Philodina*) instead of *Paramecium* (Marten [NOMCB] April 1993 p. 6). Additional foods and culture methods have been described by Wyngaard and Chinnappa (1982).

The most commonly used food sources for *Paramecium*/*Chilomonas* culture have been wheat seed or lettuce, with natural bacterial flora or an *Aerobacter* inoculum. This system is highly robust. Light or dark does not matter. Water containers should be clean, but it is not necessary to sterilize the containers or the water before use. If indoors, the production container can be left open to the air without risking invasion by microorganisms that will take over the

*Paramecium*/*Chilomonas* culture. If the container is outdoors, it is necessary to cover the container with a screen or lid to prevent invasion by aquatic insect larvae.

Continuous production has not been feasible. High yields are possible only with batch production. A typical procedure is to fill a container with charcoal-filtered tap water, add wheat seed or lettuce, and pour in a small amount of *Paramecium*/*Chilomonas* culture. A small number of adult copepods can be introduced as soon as *Paramecium*/*Chilomonas* numbers are high (typically 1 or 2 wk after introducing the *Paramecium*/*Chilomonas*). Three to four weeks later, the number of new adult copepods will be 100 times or more the original number. Most of the adults will be inseminated females.

A proper balance between the copepods and their food supply is essential for successful mass production. It is important to wait until the *Paramecium* population reaches a high level before introducing copepods. It also is important to resist the temptation to start with too many copepods. Fifty copepods in a 150-liter plastic garbage pail will produce about 10,000 new adult copepods in 3 wk. Too many will produce so many half-grown copepodids that they deplete the food supply and all die before becoming adults. Production can be improved by adding supplemental food from the time the copepodids are half-grown until the adults are removed for use. Brine shrimp are convenient supplemental food because they can be hatched from commercially available eggs.

## STORAGE AND FIELD APPLICATION

It is not practical to store a large number of copepods in a water container, because the copepods will eat one another. About half the copepods will disappear each day if they have no food. One simple solution is to lower the temperature. A hundred thousand *Macrocyclops albidus* were stored for months in a 1-liter container at 5°C (Marten 1990c). Another practical solution is to place the copepods on cubes of moist foam rubber, where they survive for months without being able to move to eat each other (Marten 1990c, Marten [NOMCB] September 1992 p 9–10, June 1993 p 6–7). Fifty copepods can be placed on a 1-cm$^2$ cube, and the cubes can be packed on top of one another in a plastic container for storage or shipment. This method is routinely used to distribute copepods in Vietnam. Putting a single cube in a water tank starts a village on its way to eradicating *Ae. aegypti*.

Introducing 10 copepods to a container habitat, large or small, is sufficient to establish a full copepod population within a month or two, but a larger number should be introduced if immediate control is desired. Introduction of 50–100 adult copepods to a tire, 100–200 to a 200-liter drum, or 1000 to a large tank will ensure full predation levels from the beginning.

A single application of *Bti* to a container at the same time copepods are introduced will help to ensure immediate control (Rivière et al. 1987a, Marten et al. 1993, Tietze et al. 1994, Kosiyachinda et al. 2003, Chansang et al. 2004). *Bacillus thuringiensis israelensis* has no deleterious effect on copepods. It will kill all of the larvae in the container at the time of copepod introduction, and the copepods can kill all newly hatched larvae after that. If *Bti* is not used, larvae that are too large for the copepods to kill may linger in the container for weeks or months and eventually emerge as adult mosquitoes. A lingering population of adult mosquitoes after copepod introduction can be removed by spraying with an adulticide that does not kill copepods (Marten 1990c). Spraying tire piles with permethrin killed the adult *Ae. albopictus* around the piles without harming *Mesocyclops longisetus* in the tires (Marten [NOMCB] August 1992 p. 7–8, October 1992 p. 6).

Copepods can be applied to one container at a time using a backpack sprayer with a nozzle that has a single hole at least 5 mm in diameter (Marten 1990c, Hallmon et al. 1993). If desired, both copepods and *Bti* can be placed together in the sprayer's tank so both are introduced to containers at the same time. Copepods can also be broadcast over a group of containers (e.g., a tire pile) using a forced-air sprayer such as an Adaptco Scorpion® (Thompson [NOMCB] July 1995, December 1995). The copepods appear to suffer no negative effects from broadcast spraying. They are deposited into the top 2–3 layers of tires, where nearly all mosquito breeding occurs. Broadcast spraying is most effective when wind is low. About 15–20% of the sprayed copepods are actually placed into the tires under these conditions. It is necessary to do 2 sprayings of about 25 copepods per tire to ensure that at least 10 copepods are introduced into 99% of the tires that are high enough in the pile for mosquitoes to be breeding in them. Marten et al. (1997) provide further procedural details.

## *COPEPODS IN SURFACE-WATER HABITATS*

Copepods naturally reduce or eliminate mosquito larvae in surface-water habitats everywhere in the world. There are numerous possibilities to enhance or add to the natural control by introducing appropriate copepod species to sites where they do not happen to be at the time. This section presents the results of field trials in temporary pools, marshes, rice fields, and roadside ditches in Louisiana.

## *CULEX* AND FLOODWATER *AEDES* IN TEMPORARY POOLS

In New Orleans it is common to find temporary pools in parks and other grassy areas such as the yards of rural homes. *Aedes sollicitans* and *Cx. salinarius* are the most common species of mosquito larvae in the temporary pools. *Aedes vexans* and *Cs. inornata* are sometimes numerous as well. *Mesocyclops* and fish that might eat mosquito larvae are never seen in these pools, but natural populations of *Diacyclops navus*, *Acanthocyclops vernalis*, and *Macrocyclops albidus* abound (Marten [NOMCB] March 1990 p 2–3).

*Acanthocyclops* and *Diacyclops* are present in large numbers in most temporary pools as soon as they have water. Because *Acanthocyclops* and *Diacyclops* that survived drying of a pool are mainly late-stage copepodids rather than adults, the ability of these 2 species to prey on mosquito larvae immediately after flooding is limited by their small size. Field surveys found no association between *Diacyclops* numbers and any species of mosquito larvae (Marten [NOMCB] March 1990 p 2–3, March 1992 p 6–7). *Aedes sollicitans* numbers are sometimes lower when *Acanthocyclops* is present, but there is no association between *Acanthocyclops* and *Cx. salinarius* larvae. *Diacyclops* and *Acanthocyclops* quickly produce large populations whenever introduced to pools in which they are not already present. They undoubtedly kill *Aedes* larvae, but their impact on larval survival is not strong enough to be of use for mosquito control.

Most temporary pools are completely dry for extended periods, but some have water or moist soil in the deepest part of the depression throughout the year. *Macrocyclops* occurs naturally only in these pools, where it has a noticeable impact on the mosquito larvae. *Aedes sollicitans* larvae are almost always absent (or present in only very low numbers) in pools with *Macrocyclops* (Marten [NOMCB] March 1990 p 2–3, March 1992 p 6–7). *Culex salinarius* is much more resistant to copepod predation than floodwater *Aedes*. *Culex salinarius* larvae are sometimes less numerous in pools with *Macrocyclops*, but the impact is not strong enough to be useful for control.

Pools with a permanent pocket of moisture but no natural *Macrocyclops* population are not uncommon. While *Mesocyclops* (*M. pehpeiensis*, *M. longisetus*, and *M. edax*) do not survive when introduced to these pools (Marten [NOMCB] May 1990 p 3), *Macrocyclops* thrives when introduced and virtually eliminates floodwater *Aedes* production thereafter (Marten [NOMCB] December 1992 p 6–7, Marten et al. 1994a). Pools that would not normally support long-term *Macrocyclops* survival because they dry out completely can be rendered more suitable for *Macrocyclops* by digging a sump hole that retains moisture through the year.

## *ANOPHELES*, *CULEX*, AND FLOODWATER *AEDES* IN MARSHES

The main mosquito larvae in Louisiana marshes are *Ae. vexans*, *Ae. sollicitans*, *Cx. salinarius*, and *An. crucians*. *Macrocyclops albidus* and *Acanthocyclops vernalis* are the large copepods that occur naturally in the marshes. The wet zones in the marshes typically expand and contract with seasonal rainfall, some marshes drying entirely at times. *Macrocyclops* predominates in *Spartina* and *Salicornia* marshes that retain moisture throughout the year. *Aedes* and *Anopheles* larvae are absent (or present in only low numbers) where *Macrocyclops* is present, but control is incomplete because *Macrocyclops* populations are patchy within a marsh. The number of *Cx. salinarius* larvae shows no relationship to the spatial distribution of *Macrocyclops* (Marten [NOMCB] November 1990 p 2–3, December 1991 p 7–8). *Acanthocyclops* predominates in marshes that sometimes dry out. There is not a strong enough association between *Acanthocyclops* and any species of mosquito larvae to suggest that *Acanthocyclops* would be useful for mosquito control.

Because marshes that dry out do not normally have a natural population of *Macrocyclops* or *Mesocyclops*, natural control of floodwater *Aedes* and *Anopheles* might be augmented by introducing these copepods into the marshes when they have water. To test this idea, 1000 *Macrocyclops albidus* and *Mesocyclops longisetus* were introduced to several points in a large *Spartina* marsh that dries out periodically (Marten et al. 1994a, Marten [NOMCB] December 1991 p 8–9). One month later, both species were numerous at distances up to several hundred meters from the points of introduction. The number of *Ae. sollicitans* and *An. crucians* larvae, which were high at the time of copepod introduction, fell nearly to zero once *M. albidus* and *M. longisetus* populations were high. *Aedes* and *Anopheles* larval numbers remained high in adjacent parts of the same marsh that served as a control without copepods. The marsh subsequently dried out, and *Macrocyclops* and *M. longisetus* did not reappear when it was naturally flooded with water again. It seems that *Anopheles* and floodwater *Aedes* production could be reduced by *Macrocyclops* or *Mesocyclops* introduction when marshes flood after drying out. There would be about a 1-month lag in control while the copepod populations build up.

## *ANOPHELES* IN RICE FIELDS AND OTHER HABITATS

A wide range of habitats was surveyed for *An. albimanus* larvae and copepods in the Atlantic and Pacific coastal zones of Colombia (Marten et al. 1989). The only large copepod species were *M. longisetus* and *M. venezolanus*. While the populations of *An. albimanus* larvae varied from absent to high at sites without large copepods, the larvae were virtually absent where large copepod populations were numerous.

A field survey in Louisiana revealed that nearly all rice fields contained *Acanthocyclops vernalis* or *Mesocyclops pehpeiensis* (then called *M. ruttneri*), though few fields contained both copepod species (Marten et al. 2000a). Only a few fields produced significant numbers of *An. quadrimaculatus* adults, and most of those fields had *A. vernalis* but not *M. pehpeiensis*. Introduction of 500 *Macrocyclops albidus*, *M. pehpeiensis*, *Mesocyclops edax*, and *Mesocyclops longisetus* to rice fields at the time of first flooding in April led to large populations of all these species 6 wk later. No *An. quadrimaculatus* larvae were seen in the treated fields from June until the fields were drained for rice harvest in August, though there were normal numbers of *An. quadrimaculatus* larvae in adjacent control fields with natural *Acanthocyclops* populations but no *Mesocyclops* or *Macrocyclops*. These results suggest that copepod introduction could substantially reduce *Anopheles* production in rice fields. The lag in the buildup of an introduced copepod population at the beginning of the rice season could be reduced by keeping a pond in each field to provide a reservoir for copepods to restock the field when it is flooded.

## *CULEX* IN ROADSIDE DITCHES

Copepods generally prey on *Culex* larvae to a lesser extent than *Aedes* and *Anopheles* larvae (Rivière and Thirel 1981, Marten 1989, Brown et al. 1991a, 1991b; Marten et al. 1994a, 2000b; Blaustein and Margalit 1994, Pérez-Serna et al. 1996, Mittal et al. 1997, Micieli et al. 2002, Soumare et al. 2004). Observations of attacks with a stereomicroscope in the laboratory (GG Marten, unpublished data) revealed that copepods lunge at *Culex* larvae as frequently as *Aedes* larvae. Whereas they usually grab *Aedes* larvae and start chewing, most attacks on *Culex* larvae are aborted upon contact. The copepod appears to "bounce off" the *Culex* larvae, only occasionally grabbing one to eat it. The explanation may lie in the more prominent spines of *Culex* larvae. Laboratory experiments of copepod predation on a variety of aquatic animals have shown they tend not to consume prey with spines (Roche 1990). Spines may make the prey more difficult to manipulate or give copepods the illusion that the prey is much larger than it really is.

Residential roadside drainage ditches in Louisiana towns provide breeding habitat for *Cx. quinquefasciatus*, particularly where the ditches are polluted by effluent from septic tanks. *Macrocyclops albidus* is the most common large copepod in the ditches, though *Acanthocyclops vernalis* and *Megacyclops latipes* are seen occasionally. While the ability of *Macrocyclops* to kill *Culex* larvae is much less than its ability to kill *Aedes* or *Anopheles* larvae, *Macrocyclops* is more effective at killing *Cx. quinquefasciatus* larvae than the larvae of other *Culex* species (Marten et al. 1994a). The interaction of *Macrocyclops* with *Cx. quinquefasciatus* larvae and mosquito fish (*Gambusia affinis*) in these ditches demonstrates how natural control of mosquito larvae by predators happens in patchy surface water habitats that change with the seasons.

The distribution of both copepods and mosquito fish along the ditches is shaped by the fact that neither copepods nor fish can live in the highly polluted water typically found within 5–10 meters of septic tank outlets (Marten et al. 2000b). Mosquito fish spread through unpolluted parts of the ditches during late spring and early summer but disappear from most of the ditches when the weather turns cold in late autumn. The distribution of *Macrocyclops* tends to complement mosquito fish because the fish eat copepods. *Macrocyclops* starts to spread through unpolluted parts of the ditches during the autumn when fish are in decline and is common throughout the ditches by spring. It then disappears from many parts of the ditches during the summer because mosquito fish are expanding through the ditches, water temperatures are too high for *Macrocyclops*, and pollution is more severe due to reduced water flows and shallow water during the summer.

Mosquito fish reduce *Cx. quinquefasciatus* production to virtually zero wherever they are present. Copepod predation is less absolute. *Macrocyclops* has a fill-in role for natural *Cx. quinquefasciatus* control by occupying many parts of the ditches when fish are not there. There were about 90% fewer *Cx. quinquefasciatus* larvae in stretches of the ditches with a natural *Macrocyclops* population (and no fish) compared to stretches where neither predator was present (Marten et al. 2000b). In field experiments to assess larval survival after introducing several thousand *Cx. quinquefasciatus* larvae as egg rafts, 2.6% of the larvae survived to the 4th instar where *Macrocyclops* was naturally present (without fish), compared to 46% survival where neither fish nor *Macrocyclops* were present.

*Culex quinquefasciatus* production is often enormous in the polluted water near septic-tank outlets where copepods and mosquito fish cannot live. There can also be *Cx. quinquefasciatus* production in unpolluted water if neither copepods nor mosquito fish are present. This happens from October to March, when mosquito fish have disappeared but *Macrocyclops* has not yet filled the ditches in the course of its seasonal expansion. This is a time when natural control is low. It is also a time when natural control can be augmented by introducing *Macrocyclops* throughout the ditches. In a field trial to test this idea, introduction of *Macrocyclops* to the ditches in October reduced the number of sites with *Cx. quinquefasciatus* larvae by 75% during November to March compared to ditches without *Macrocyclops* introduction (Marten et al. 2000b).

## *ENVIRONMENTAL AND HEALTH IMPACTS*

### ENVIRONMENTAL IMPACTS

The use of copepods for mosquito control has no significant undesirable environmental impact. As broad-spectrum predators, copepods dramatically reduce the populations of many species of small aquatic animals in artificial container habitats (Rivière 1985). This is of no consequence to the natural environment. Copepods also impact small aquatic animal populations when introduced to temporary pools, marshes, rice fields, or other surface water habitats, but as long as local copepod species are used for the introductions, the outcome is no different from what already happens in numerous sites in the same area that already have natural copepod populations.

There is no need to use exotic species of copepods for mosquito control. Almost everywhere, there is a local species available to do the job. "Local species" can be any that are found in the same ecological region. Copepods cultured from collection in one country should be appropriate for use in another country as long as it is in the same ecological region.

### HEALTH IMPACTS

Some species of copepods are known to be intermediate hosts for guinea worm (*Dracunculus medinensis* Linnaeus) where this human parasite is present in West Africa and South Asia (Muller 1991, Cairncross et al. 2002, Hopkins et al. 1995). Guinea worm larvae are eaten live by copepods and enter humans when they swallow copepods in drinking water. The larvae develop into a worm that can exceed a meter in length, rupturing the skin to lay eggs when a person bathes the sore in water. Infection of the sore can be seriously disabling for several months.

Guinea worm larvae appear to have little host specificity among copepod species. Species that have been found with natural infections include *Mesocyclops aequatorialis*, *M. kieferi* Van de Velde, *Thermocyclops decipiens* (Kiefer), *T. incisus* (Kiefer), *T. inopinus* (Kiefer), and *T. nigerianus* Kiefer (Anosike et al. 2003, Okoye et al. 1995, Steib and Mayer 1988, Yelifari et al. 1997). The most important intermediate hosts seem to be those large copepods that predominate in ponds where they are likely to be ingested by people.

There is no hazard from guinea worms outside the limited geographic areas where they occur. Where they do occur, the hazard is low because guinea-worm eradication programs during recent years have taken this parasite close to eradication. In those few areas where guinea worm still exists, the hazard can be eliminated by not bathing guinea-worm sores in drinking water where copepods are used for mosquito control and by filtering drinking water through a cloth to remove copepods before consuming the water.

There has been a concern in recent years that copepods may facilitate cholera transmission (Reidl and Klose 2002, Gonçalves et al. 2004). The bodies of copepods and other planktonic animals provide a surface for bacteria and bacteria can live in the gut (Zampini et al. 2005). The practical significance remains uncertain. On one hand, lower cholera rates were associated with community field trials filtering copepods from drinking water in Bangladesh (Colwell et al. 2003). On the other hand, there has never been a problem with cholera when copepods have been used for mosquito control in water storage containers, including thousands of households in Vietnam. A laboratory study in Brazil has shown that cholera bacteria cannot survive in the water where copepods would be used to control container-breeding mosquitoes (Araújo et al. 1996). When inoculant from a cholera culture was added to a container with water from a reservoir (pH = 6.5), all viable cholera bacteria disappeared within a day, regardless of whether *Mesocyclops longisetus* was in the water. It took a week for the cholera to disappear from the water at pH 7.5. If *M. longisetus* was in the water at pH 7.5, viable cholera cells could be cultured from their bodies, but the cholera lasted in the water only a day longer than in water without copepods. In any event, filtering copepods and other small aquatic animals from water before drinking should be standard procedure because their body surfaces may harbor other bacteria such as *Enterococcus faecalis* (Signoretto et al. 2005).

## *GETTING STARTED*

### SETTING UP LOCAL SPECIES CULTURES

The first step is to set up cultures of all large copepod species in the area. This is best accomplished by collecting large copepods from

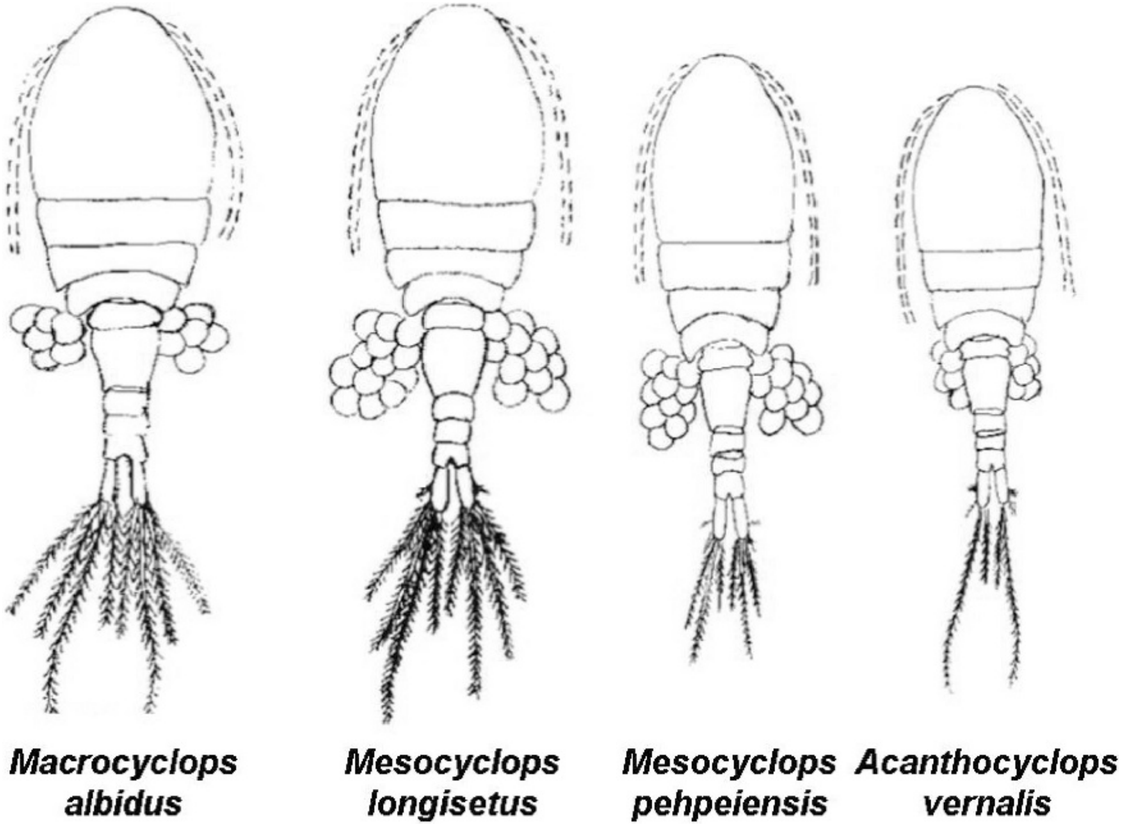

Fig. 6. Gross morphology of some common larvivorous copepods in Louisiana. Source: Marten et al. (1997).

as many different local aquatic habitats as possible. Local species are the best candidates for mosquito control because they are adapted to the local climate and hydrological conditions.

The easiest way to collect the copepods is to scoop water from a collection site with a bucket or larval dipper and pour it through a piece of plankton netting (200 micron mesh). The netting can be sandwiched between 2 kitchen strainers – one strainer underneath the net to hold it and the other strainer on top of the net to strain out debris. After pouring water through the net, captured animals can be transferred to a container of water by inverting the net into the water and shaking it gently. If the water is tap water, it should be charcoal filtered or exposed to the air for a few days to remove chlorine before use. Traps are another way to collect copepods (Kay et al. 1992a, Gionar et al. 1999).

Some of the specimens from field collections can be preserved in small vials of alcohol for identification. Most should be used to start single-female cultures in small laboratory containers. Culture techniques can be the same as those used for mass production (Suárez et al. 1992, Marten et al. 1997). Because different copepod species can be so different in their performance, accurate identification is necessary not only during the initial field-survey stage but also later to monitor established cultures against contamination by other species.

Taxonomic understanding has been refined considerably during the past 2 decades. Entrées to the taxonomic literature and explanations of taxonomic technique can be found in Dussart and Defaye (2001), Einsle (1993, 1996), Williamson and Reid (2001), Ueda and Reid (2003), Boxshall and Halsey (2004), and Hołyńska (2006). Recent identification manuals are available for the most important genera: *Macrocyclops*, *Megacyclops* (Einsle 1993, 1996), and *Mesocyclops* (Ueda and Reid 2003). Because the key characters for species identification involve small differences in the proportions of certain body parts and morphological "microcharacters" such as spines, setules, or processes on the bodies, it is essential to have technical support from a specialist on copepod taxonomy. The fact that these key characters can be seen only by dissecting the specimen means that initial species identification is only possible with dead specimens.

Aggregated cultures for each species can be set up after identifying a few specimens from each single-female culture and pooling all cultures of

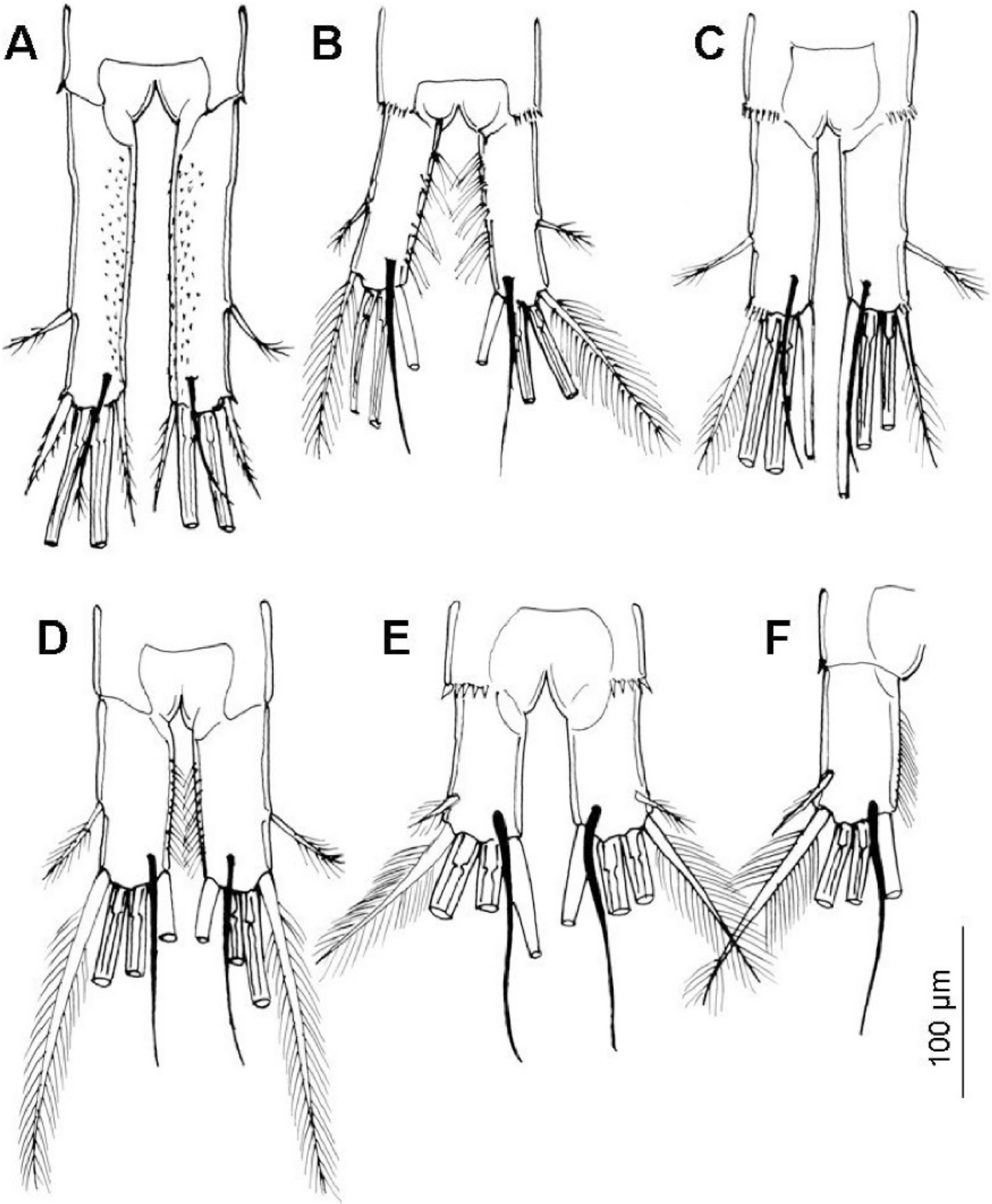

Fig. 7. Close-up view of caudal rami and setae of some common larvivorous copepods. A. *Acanthocyclops vernalis*; B. *Mesocyclops edax*; C. *Mesocyclops pehpeiensis*; D. *Mesocyclops longisetus*; E. *Macrocyclops albidus*; F = *Macrocyclops fuscus*. Source: Janet Reid.

the same species. Once the species identity of each culture has been reliably ascertained from conventional key characters, the animals in the cultures can be examined for ways to identify the species of live copepods, using their behavior and gross morphology (Fig. 6). Figure 7 shows how easily seen differences in the shape and proportions of the caudal rami can be used to distinguish the most important North American species. The swimming behavior of live copepods can also be used to tell them apart. Some species swim in the middle of a laboratory container, while others concentrate near the bottom or cling to the sides.

## ASSESSING THE EFFECTIVENESS OF DIFFERENT COPEPOD SPECIES

The next step after setting up local species cultures is a quick laboratory assessment of the strength of each species as a predator. The basic procedure is to count how many 1st instars a single copepod kills over 24 h with a surplus of larvae in a small container (e.g., 10 ml of water). Fifty larvae per container are usually sufficient. Because variation from replicate to replicate can be large, it is best to run at least 50 replicates for each combination of copepod species and mosquito species to secure a reliable average. Any copepod species that kills an average of 40 or more larvae is a strong candidate for biological control.

It is then necessary to check the best copepod species from the laboratory experiments for their survival in container habitats where they might be used. Field experiments are essential because the fit of different species to different habitats can be subtle and not readily predicted. The only way to know for sure how many copepods of a particular species a particular habitat can sustain, how long the copepod population will persist after introduction, and how effectively the copepods reduce larval survival is to see what happens after introducing some copepods. An effective species will establish and maintain a population of more than 50 adults in tires, more than 500 in a 200 ml drum, and several thousand in a larger water-storage tank. The best copepod species for introduction into containers may not be common in nature. *Mesocyclops longisetus* has proved the most effective species for tires in Louisiana, even though it is rarely found in natural habitats there (Marten 1990b, Marten et al. 1994a).

## *CONCLUDING REMARKS*

Matching copepods to appropriate mosquito breeding habitats is one of the keys to using them effectively. The other side of the coin is recognizing habitats for which copepods are not effective and dealing with those habitats by other means. Citizen participation is a key ingredient for using copepods against *Ae. aegypti* and *Ae. albopictus* in villages or urban residential areas. It is generally beyond the capacity of governments or other outside agencies to maintain copepods in containers scattered through people's yards. It is often difficult, but not impossible to organize citizen participation where it does not already exist for some other purpose.

Recalling the "egg trap effect" described earlier in this chapter, converting a breeding site to an "egg sink" is more effective for mosquito control than eliminating the site. This perspective can be extended to situations with an abundance of breeding sites that are hidden or otherwise not eliminated by source reduction. A possible future use of copepods is as the "larvicide" in egg traps that are put out to compete with breeding sites that remain after source reduction. The necessary number of egg traps would be substantial, probably outnumbering existing breeding sites by at least 5–1 (Nam et al. 1998). This is a practical possibility with community participation.

The rewards from copepods can be substantial. Where appropriate, they offer reliable, long-term, and environmentally friendly control while saving money on insecticide use. However, like all other forms of biological control, copepods are far from free. To be of practical value they require as much attention, effort, and budget support as any other control method. The main costs of copepod use are associated with professional inputs:

- identifying appropriate habitats for copepod use;
- working out exactly how to use them;
- mass producing the copepods;
- designing integrated control programs in which copepods have a role;
- organizing community participation;
- adaptively sustaining the program.

Until now, limitations in this kind of professional capacity have been a serious obstacle to large-scale copepod use. Building up this kind of professional capacity will be necessary if copepods are to become a more common part of the mosquito-control arsenal.

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