Copepod Production
&
Application
for
Mosquito Control

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Produced for
New Orleans Mosquito Control Board
City of New Orleans

1997

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INTRODUCTION

Cyclopoid copepods (also known as "cyclops") are tiny crustaceans that prey on many species of mosquito larvae. As part of an ongoing program to develop alternatives to pesticides for mosquito control, the New Orleans Mosquito Control Board employs *Mesocyclops longisetus* and *Macrocyclops albidus* in control operations for *Aedes* larvae in container and floodwater habitats. Field trials conducted by the New Orleans Mosquito Control Board have demonstrated that cyclops can contribute to an integrated program for control of *Culex quinquefasciatus* and *Anopheles* larvae in habitats such as residential ditches, marshes, and ricefields.

Cyclops are exceptionally effective predators for the control of mosquito larvae because:

1. When introduced to most aquatic habitats, they multiply to large numbers and maintain those numbers for as long as there is water or moisture.
2. They kill most mosquito larvae, often eliminating virtually all larvae that hatch into the habitat.
3. They can be mass produced at low cost.
4. They are easily transported for introduction to mosquito breeding sites.

This manual provides a step-by-step description of procedures followed by the New Orleans Mosquito Control Board for the mass production and deployment of cyclops. It starts with field collection of local species of cyclops to set up single-species cultures. It then describes how to identify cyclops, how to maintain cyclops "stock cultures" in five-gallon bottles, and how to evaluate each species of cyclops for mosquito control. The manual finishes with a description of how we apply cyclops to tires and temporary bodies of water (swales). Cyclops survive application from sprayers, so they can be applied to large numbers of tires at a relatively low labor cost per tire.

The heart of the manual deals with mass production, and the key to mass production of cyclops is their food supply. We hoped to use prepared foods. However, after testing a great variety of prepared and live foods, we were only able to produce large numbers of cyclops with live food. We use two kinds of protozoa: *Chilomonas* (tiny one-celled flagellates), which are food for cyclops during their early stages of growth, and *Paramecium caudatum* (larger one-celled organisms), which are food for half-grown juvenile to adult cyclops.

Our mass production system is an indoor batch system that uses shallow fiberglass trays stacked in racks. The trays are shallow to maximize the air-water interface for

oxygen exchange. After establishing a dense population of *Chilomonas* and *Paramecium* in a tray, we introduce an adult female cyclops from a single species. We harvest more than one hundred times as many adults four weeks later.

The indoor batch system is simple and reliable, providing high yields of pure cyclops on a sustainable basis. After extensive testing, we chose not to use outdoor tanks or ponds, which in theory could produce a continuous harvest of cyclops. Yields from outdoor tanks and ponds are usually low for two reasons. One, it is not possible to maintain high quality cyclops food in the water. Two, other kinds of aquatic animals invade and compete with cyclops for the food. A mixture of other animals with cyclops may be detrimental when applying cyclops for mosquito control in the field.

COLLECTING CYCLOPS IN THE FIELD

Two different procedures can be used to collect cyclops from the field. It is better to use Procedure 1 if cyclops are plentiful in the water or only a small number are needed. The advantage to this procedure is that the cyclops collected are relatively free of debris. It is better to use Procedure 2 if only a few cyclops are present in the water or many cyclops are needed.

The two procedures are outlined below:

Procedure 1:

Materials needed

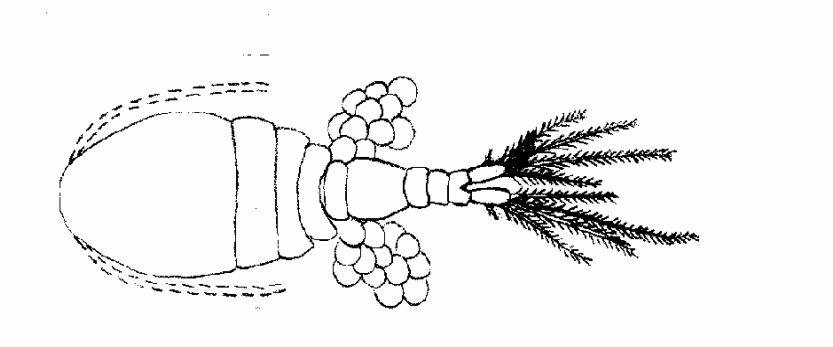
Standard dipper for mosquito larvae
0.5-gal. plastic food containers with lids
Plastic Nalgene bellows pipette (or similar pipette >5" in length)
Labeling tape (3/4" or wider)
Black Sharpie pen (or other pen with waterproof ink)
Glass vial with tight fitting screw cap

Collect water from around submerged vegetation using the dipper. Scoop the water in a way that will cause a rapid flow of water into the dipper. (Cyclops are remarkably strong swimmers and will escape from a dipper filled too slowly.)

After each dip, slowly pour about 75% of the water out of the dipper onto the ground. (This reduces the amount of water in the dipper and makes it easier to see if there are cyclops in the dip. Cyclops will not be poured out of the dipper because they will swim against the current.)

ADULT CYCLOPS

If there are cyclops in the dipper, quickly dump the remaining water, which contains the cyclops, from the dipper into a plastic container.



(If you pour slowly, the cyclops will be stranded in the dipper.)

Add a strip of labeling tape to the side of the plastic container and note the date and collection location.

Cover the container with a lid.

CAUTION! Do not place the container in the sun while making additional collections or during transport. Cyclops are hardy creatures, but are unable to withstand the high temperature created by placing a closed container in the sunlight.

After taking the containers to the laboratory, remove several of the cyclops with a pipette and place them in (a) a vial of > 70% alcohol for preservation and identification (See Cyclops Identification, p. 8), or (b) a culture dish for single-female culture (See Starting Cyclops Cultures From Field Collections, p. 6).

NOTE: The alcohol placed in a vial for preservation should not be diluted to less than 70% strength. One way to ensure this is to use a bulb pipette to put cyclops in a vial without alcohol. Once all the cyclops are in the vial, use the pipette to draw water slowly from the vial, drawing from the top of the water. The cyclops should remain in the vial, and once the water is reduced to <20% of the total capacity of the vial, the vial can be filled with alcohol.

Label the vial or culture dish as to the collection date and location.

Procedure 2:

Materials needed
Standard larval dipper
Netting (200 um mesh)
0.5-gal. plastic food containers with lids
Non-chlorinated water
Pipette (5" or longer)
Two metal strainers (the kind used in kitchens)
Two-gallon bucket
Labeling tape (3/4" or wider)
Black Sharpie pen

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Spread the netting across the top of one strainer.

Place a second strainer into the netting-covered strainer (like two stacked spoons) resulting in the netting being held like meat between two slices of bread in a sandwich.

Scoop water from the collection site with a dipper or a bucket, and pour the water through the strainers. (The strainer will catch most of the debris while allowing the cyclops to pass through.)

Remove the top strainer and place aside when enough scooping has been completed to assure the collection of sufficient cyclops for your needs. (The number of cyclops collected will, at this point, have to be a guess.)

Carefully remove the netting. (Any adult cyclops present in the water sample should have been caught by the netting.)

To transfer the cyclops, invert the net, and dip it into a two-liter plastic container that contains approximately one liter of non-chlorinated water. Move the inverted net laterally back and forth in the water until all of the cyclops appear to be shaken free from the net.

IMPORTANT! Two methods are available for removing chlorine from tap water: 1) neutralizing the chlorine by adding a chemical to the water, and 2) filtering the water through an industrial charcoal filter.

NOTE: The treatment of tap water with sodium thiocyanate drops, which remove chlorine and chloramine, is a very cost-effective method, but the use of a charcoal filter is more practical since it can treat thousands of gallons of water and is immediately available in the laboratory at all times.

Attach a strip of labeling tape to the container and process it in the laboratory in the same manner as in Procedure 1.

STARTING CYCLOPS CULTURES FROM FIELD COLLECTIONS

Because cyclops species differ in their capacity as predators of mosquito larvae and in their ability to survive in various kinds of aquatic habitats, it is important that all cyclops used in field trials or mosquito control operations come from single-species cultures. The best way to establish a single-species culture is to start one from a single female. Fertilization of female cyclops takes place just after the adult molt. The females produce a series of egg clutches from the single fertilization (a new clutch every 5-7 days). Few adult males or unfertilized females are collected from nature. For this reason, any adult cyclops collected in the field is probably a fertilized female that will produce offspring when placed in a food rich environment.

Materials needed

Two glass dishes (approximately 4 1/2" in diameter and 2" deep)
Autoclaved wheat seeds
Non-chlorinated water
Chilomonas culture
Paramecium culture
Plastic Nalgene bellows pipette
Labeling tape
Sharpie pen

Procedure:

Pour approximately 250 ml of non-chlorinated water into one of the dishes.

Add the following:

Four autoclaved wheat seeds. (Using sterilized seeds will greatly reduce the possibility of undesirable bacteria and fungus growing in the cultures.)
(For the 99% of us who do not have a ready supply of wheat seeds,

Carolina Biological Supply sells seeds sterilized by autoclaving. Otherwise wheat seeds can be sterilized by dropping the seeds into boiling water, draining after one minute, and immediately using the seeds. If this technique is used, be certain that the seeds have not been treated with pesticides or fungicides.)

NOTE: Other grains (e.g., rice) can be used. We prefer wheat because other grains disintegrate into fine debris that is difficult to separate from the cyclops when they are harvested.

Approximately 20 ml of *Chilomonas* culture by pouring from a culture flask (See Culturing *Chilomonas,* p. 12).

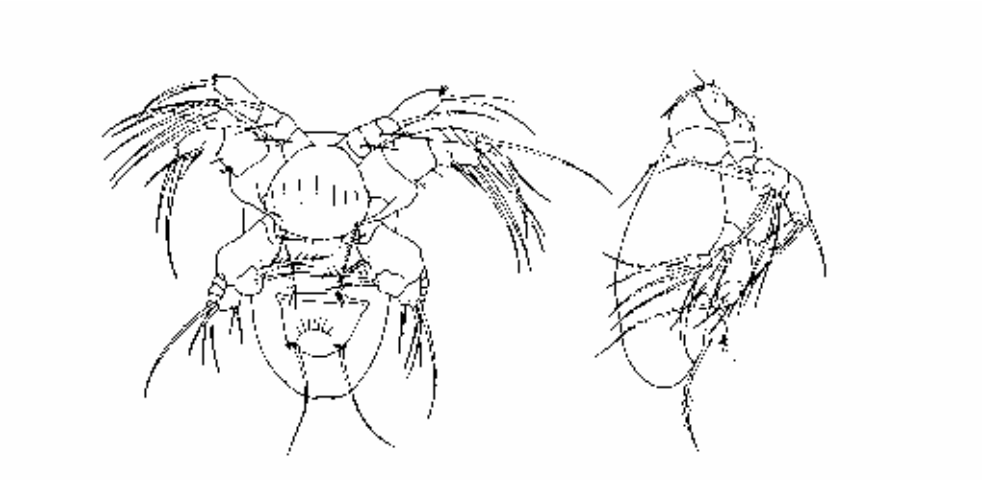
Twenty ml of *Paramecium* culture also by pouring from a culture flask (See Culturing *Paramecium,* p. 14).

Use a pipette to capture a single egg-bearing female and place her and accompanying drop of water into bottom of a the second dish.

Observe this drop under a stereomicroscope to make sure there are no copepodids or nauplii are also in the drop. (If the single cyclops has unwanted company, use the pipette to return everything to the original container. Rinse out the dish, dry, and try again.)

IMPORTANT! Make sure that no small, immature cyclops (copepodids or nauplii) are transferred along with the single adult. (Nauplii are the first stage after cyclops hatch from the eggs that females carry in the sacs at the sides of their body. They do not have tails and look somewhat like tiny ticks. Copepodids are the stage between nauplii and adults. Unlike Nauplii, copepodids have tails and look similar to the adults, except that they are smaller.)

NAUPLII



Fill the dish containing the cyclops about halfway with non-chlorinated water.

Recapture the egg-bearing female with the pipette and place her into the dish containing wheat seeds, *Chilomonas*, and *Paramecium*. (The two dishes are used to prevent any chance of immature cyclops being transferred to the culture dish with the single adult female.)

Label the dish.

The developing cyclops cultures may be kept in an uncovered dish. They do not require light, but the temperature must be maintained between 20°and 30°C (68°and 86°F).

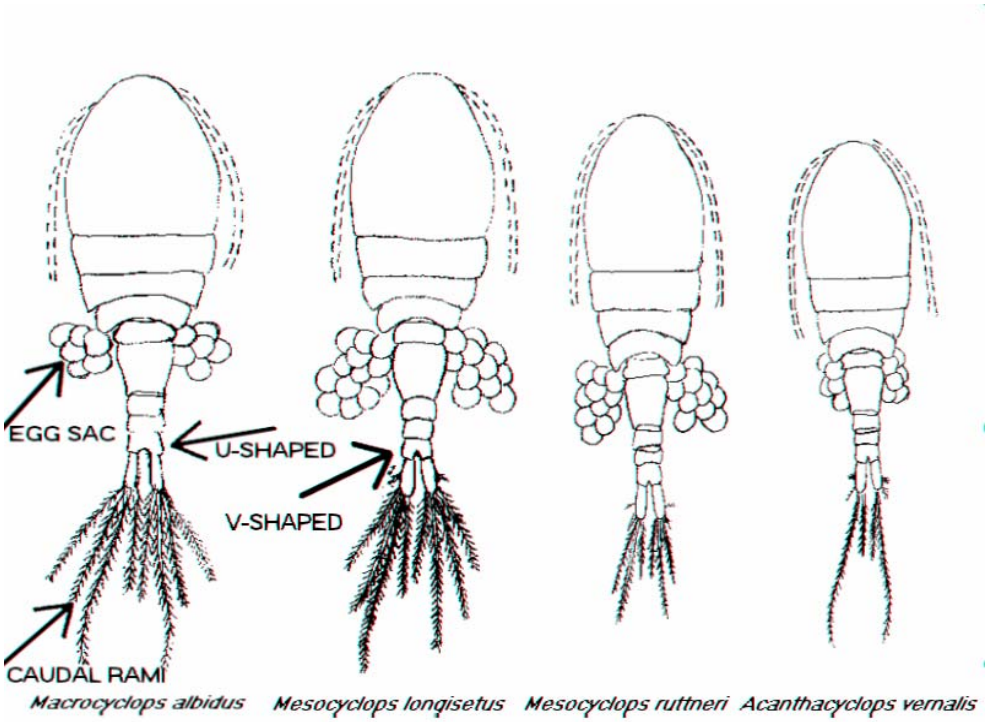
Nauplii should hatch from the eggs in the female's eggsacs within a few days and they should grow to mature adults within two weeks.

Several mature adults from each culture can be preserved in alcohol and mailed away for positive identification (See Production Suppliers, Appendix III). Adult females are normally used for species identifications.

CYCLOPS IDENTIFICATION

Reliable identification of cyclops not already well known to the observer is possible only by means of key characters examined on preserved specimens. Only specialists in cyclops taxonomy can do such identifications with complete confidence. Dr. Janet Reid e Production Suppliers, Appendix III) has provided cyclops identifications for the New Orleans Mosquito Control Board.

Once the cyclops have been positively identified, a stereomicroscope can be used to distinguish living specimens on the basis of their external characteristics. The most useful parts of the body for live identifications are the egg sacs and caudal rami (See illustrations below). When viewed under a stereomicroscope:



Macrocyclops albidus: This cyclops is slightly blue green and darker in color than *Mesocyclops*. The body is fatter and the caudal rami are shaped more like a U than a V. Each caudal ramus has four long caudal setae. The egg sac is shorter and fatter and contains fewer eggs.

Mesocyclops longisetus: The body is longer than that of *Macrocyclops* and the caudal rami form a V shape. Each caudal ramus has four long caudal setae. The transparent egg sac is long, with more eggs per egg sac than *Macrocyclops* or

Acanthocyclops.

Mesocyclops ruttneri. Looks similar to *Mesocyclops longisetus*, except it is slightly smaller and the caudal setae are shorter than the caudal section "abdomen" of the body.

Acanthocyclops vernalis: The body is smaller *Macrocyclops* and *Mesocyclops*. Each caudal ramus has only two long caudal setae. These cyclops hold their eggs sacs close to their bodies while swimming.

It is easiest to see these features if cyclops movement is reduced by lowering the water temperature to approximately 13-16°C (56-61°F).

Materials needed

Small glass dishes (approximately 1 1/2" in diameter and 1" deep)
Large glass dishes (approximately 4 1/2" in diameter and 2" deep)
Non-chlorinated water
Ice
Stereomicroscope
Fiber optics illuminator

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Procedure:

Place live cyclops in a small amount of non-chlorinated water in a small dish.

Place ice and a small amount of water in a large dish.

Set the small dish on top of the ice slush in the large dish, and allow it to cool for a few minutes.

Observe the cyclops under a stereomicroscope at 12x-50x magnification, using side illumination from a fiber optics illuminator.

SELECTING CYCLOPS SPECIES TO USE FOR MOSQUITO CONTROL

Once single-species cultures are established, they can be tested to determine which species are best for specific species of mosquito larvae in specific breeding habitats. The first step is to test the cyclops in the laboratory.

Place ten cyclops in a plastic or glass container (250-500 ml capacity) that is half-full with non-chlorinated water.

Place 500 newly hatched larvae of a single mosquito species in the container and keep at an appropriate temperature for 24 hours.

After 24 hours, count the number of surviving larvae in the container. If all larvae are dead, repeat the procedure with five cyclops in the container instead of ten.

Cyclops species that kill more than 30 first instar mosquito larvae in 24 hours are excellent candidates for mosquito control. The following procedure can be used to test them in the field:

Introduce cyclops to at least 50 containers or 25 groundwater sites. Twenty-five cyclops should be introduced to each container, and 500-1000 cyclops should be introduced to each groundwater site.

Sample the site monthly to determine whether the introduced cyclops are still there and to observe whether there are mosquito larvae.

IMPORTANT! The effectiveness of cyclops in controlling mosquitoes is dependent on how well the cyclops survive under field conditions.

OVERVIEW OF CYCLOPS PRODUCTION

Copepods are hardy animals capable of adapting to a wide range of rearing conditions as long as food is present.

Our biological control laboratory contains fifty 4'x8' fiberglass trays stacked in racks of five trays to a rack. Each tray can produce about 30,000 adult *Mesocyclops* or 20,000 *Macrocyclops* every two months. Full production yields 500,000-750,000 cyclops ready for distribution each month.

The following rearing procedure is the batch production system that we have found to work best for large-scale cyclops production.

Before beginning initial cyclops production, it will be necessary to order *Chilomonas* sp. and *Paramecium caudatum* cultures (See Production Suppliers, Appendix III). It will also be necessary to obtain the appropriate species of cyclops from a supplier or to collect cyclops from the field (See Starting Cyclops)

Cultures from Field Collections, **p. 6**). Seed cultures of all of these organisms will be available "in house" once cyclops production is under way.

Our cyclops production system is based on four subsystems that are operated in parallel

and feed into one another. The production of cyclops from initial culture establishment to use is outlined as follows:

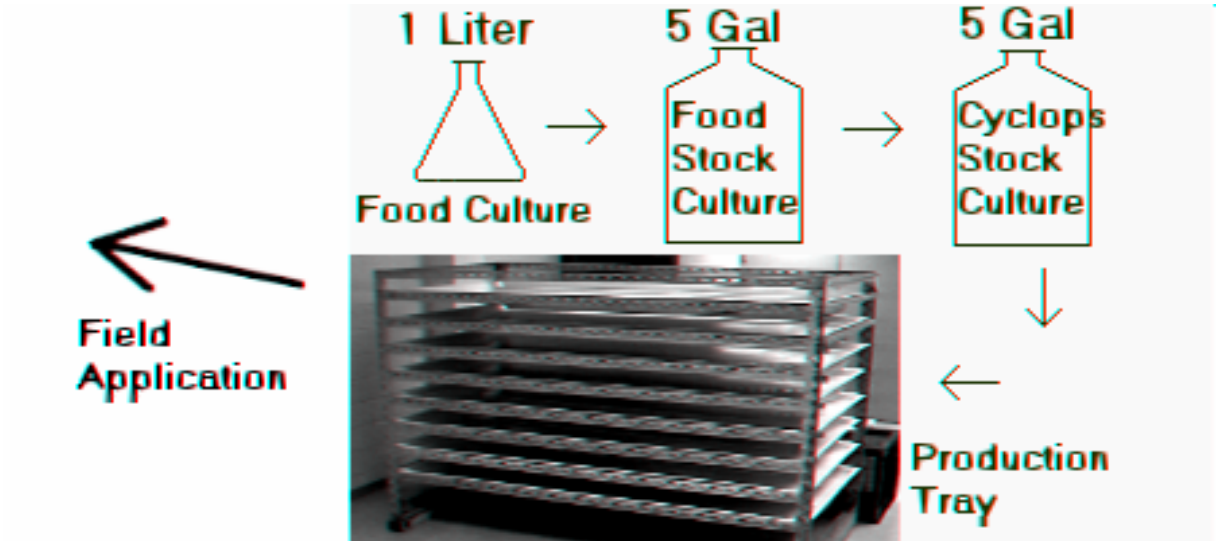
1. One-liter flasks of cyclops food (*Chilomonas* and *Paramecium*). The flasks provide *Chilomonas* and *Paramecium* for stock cultures of cyclops food. Flasks can be operated on a cycle as short as two weeks or as long as two months.

2. Five-gallon bottles of food stock cultures (*Chilomonas* and *Paramecium*). These larger containers provide food for cyclops stock cultures and for cyclops production trays. Food stock cultures can be operated on a cycle as short as two weeks or as long as two months.

3. Five-gallon bottles of cyclops stock cultures. Cyclops stock cultures are also maintained in five-gallon bottles. They provide cyclops for introduction to production trays. Cyclops stock cultures can be operated on a cycle as short as five weeks or as long as two months.

4. Cyclops production trays. Production trays provide cyclops for use in mosquito control. The entire production cycle requires about six weeks:
   a. Cleaning a tray, introducing cyclops food, and allowing the food to multiply to full strength (2-3 weeks).
   b. Introducing cyclops and allowing the next generation to grow to maturity (3-4 weeks).

Once a new generation of cyclops has reached maturity, it can be kept in a production tray for up to two months (with supplemental feeding) before removal for use in mosquito control.



CULTURING *CHILOMONAS*

Materials needed
Chilomonas source culture
One-liter Pyrex flask
Autoclaved wheat seeds
Aluminum foil
Autoclave or hot water [>70°C (>170°F)]
Non-chlorinated water
Labeling tape
Sharpie pen

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If an existing source culture is not available, order a *Chilomonas* culture from a scientific supply house (See Production Suppliers, Appendix III).

Procedure:

Begin with a sterilized one-liter Pyrex flask.

Sterilizing a flask

Use a bottle scrubber and hot water to flush the interior of the flask. If available, place the flask in an autoclave to sterilize. If an autoclave is unavailable, fill the flask to its rim with hot water [>70°C (>170°F)]. Cover the top of the flask with aluminum foil, and permit the water to cool for about one hour before emptying.

Add 0.5 liters of non-chlorinated water to the sterilized flask.

Add ten wheat seeds.

Allow the water to reach room temperature by letting the flask sit for at least two hours before proceeding.

Pour about 50 ml of *Chilomonas* culture from an existing culture or from a purchased starter culture into the flask.

Cover the neck of the flask with aluminum foil.

Place a strip of labeling tape on the side of the flask. Use a Sharpie pen to label the flask with the date and the ingredients of the culture.

Keep in a dark place (such as inside a cabinet) to prevent algal growth and to speed multiplication.

CAUTION! Never place the culture in a refrigerator or in direct sunlight. The ideal temperature for multiplication is 20°C to 22°C (68°F to 72°F).

A flask culture of *Chilomonas* should reach maximum population numbers in 1-2 weeks.

A high density of *Chilomonas* can be maintained for several months by adding three autoclaved wheat seeds to the culture each week.

NOTE: Seeds will support the growth of fungal vegetative filaments that look like cotton. While unattractive, this is a normal feature of a healthy culture.

IMPORTANT! When pouring the contents of a *Chilomonas* culture flask into a new start-up flask or into a five-gallon bottle to start a stock culture (as described below in Preparing Stock Cultures, p. 17), leave 50 ml of the liquid in the original flask to provide the start up for a continuing *Chilomonas* culture in that flask. The flask culture can be restarted by simply refilling the same flask with non-chlorinated water, adding ten additional wheat seeds, covering the top with foil, attaching a new label, and returning it to storage.

CULTURING *PARAMECIUM*

Materials needed

Paramecium caudatum source culture
Chilomonas source culture
Two-liter Pyrex flask
Autoclaved wheat seeds
Aluminum foil
Non-chlorinated water
Sterile pipette
Labeling tape
Sharpie pen

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Procedure:

If an existing source culture is not available, order a *Paramecium caudatum* culture from a scientific supply house (See Production Suppliers, Appendix III) NOTE: These are non-photosynthetic *Paramecium* that do not require light to grow and reproduce.

Sterilize a two-liter Pyrex flask (See Culturing *Chilomonas*, p. 12).

Add one liter of non-chlorinated water to the two-liter flask.

Add 15 autoclaved wheat seeds.

Set the flask aside for one hour. This will permit the water to warm to room temperature.

Add about 50 ml of *Chilomonas* culture and 50 ml of *Paramecium* culture.

Cover with foil.

Attach a strip of labeling tape to the flask and record the date and the flask's contents.

Store in cool dark place for one week. [Ideal temperature for multiplication is 20°C to 22°C (68°F to 72°F). *Paramecium* cultures do not function well at temperatures above 30°C (86°F).]

CAUTION! Never place a *Paramecium* culture in the refrigerator or in direct sunlight.

What happens in the Flask? The wheat seeds serve as food for bacteria, the bacteria serve as food for the *Chilomonas*, and the bacteria and *Chilomonas* serve as food for the *Paramecium*. Both bacteria and *Chilomonas* are already present in ongoing cultures of *Paramecium*. However, separate culturing of *Chilomonas* permits their rapid multiplication to maximum numbers.)

After two weeks, the colony should have reached its maximum population.

Problem: If the concentration of *Chilomonas* or *Paramecium* is not high, add several more wheat seeds and several additional ml of *Chilomonas* and *Paramecium* culture (See Checking Population Levels, p. 16). Cover the top of the flask with foil, update the label, and place in storage for one more week. If the population levels are still not high, discard the contents of the flask and start anew.

High *Chilomonas* and *Paramecium* densities can be maintained in the flask for an additional two months by the weekly addition of three wheat seeds.

IMPORTANT! Before using the contents of a flask containing *Chilomonas* and/or *Paramecium* for cyclops production, remember to save about 50 ml of the flask's liquid to start a replacement culture.

CHECKING POPULATION LEVELS

Materials needed

Flask of *Chilomonas* or *Paramecium* culture to be checked
1 1/2" diameter Pyrex dish (or other small culture dish)
Stereomicroscope

Procedure:

Gently tip the bottle or flask over far enough to pour a small amount of liquid into the dish.
Examine under a stereomicroscope to determine whether the concentration of the desired organism is high, medium, or low.

A high density of *Paramecium* is 800 to 1,000 per ml or more.

A high density of *Chilomonas* is an uncountable number. With experience, it is easy to tell whether the concentration of *Chilomonas* or *Paramecium* is high, medium, or low.

PREPARING STOCK CULTURES

The purpose of stock cultures is to maintain large pure sources of *Chilomonas*, *Paramecium caudatum*, and cyclops for use in production trays.

Preparing a stock culture of *Chilomonas*

Materials needed
One-liter flask of *Chilomonas*
Five-gallon glass bottle
Long-handled scrub brush (26" or longer)
Plastic funnel
Hot water [>70°C (>170°F)]
Autoclaved wheat seeds
Non-chlorinated water
Aluminum foil
Labeling tape
Sharpie pen

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Procedure:

Place a five-gallon bottle where it can be filled with very hot water. (Placing the bottle where it can be filled using an overhead sprayer is ideal.)

Place a funnel into the bottle top.

Pour the hot water into the funnel. **CAUTION**! If a sprayer is used, hot water may spray upward if the sprayer is turned on too strongly.
Add about 2.5 gallons of hot water to the bottle.

Thoroughly scrub the interior of the bottle using a long-handled brush. (The brush needs to be at least 26" long to reach all internal bottle surfaces.)

IMPORTANT! Use only water. Soaps or other cleaning solutions can leave

residues that are harmful to *Chilomonas*, *Paramecium*, and cyclops.

Empty bottle. **CAUTION!** Bottle and water will be very hot.

Fill the bottle with hot water. This time fill the bottle completely.

Cover the neck of the bottle with aluminum foil and permit the water to cool for about four hours.

Drain the bottle after it cools.

Add about 2.5 gallons of non-chlorinated water to the bottle.

Permit the water to reach room temperature by letting the bottle sit for at least three hours.

Place a funnel into the neck of the bottle, and add 70 wheat seeds.

Add most of the contents of a one-liter flask of *Chilomonas* (See Culturing *Chilomonas*, p. 12). **IMPORTANT!** Do not pour out the entire flask of *Chilomonas.* Leave about 50 ml of fluid to serve as the seed for a continuing pure *Chilomonas* culture.

Cover the top of the bottle with aluminum foil.

Attach a strip of labeling tape to the bottle. Record the date and the contents of the bottle.

Take the flask still containing about 50 ml of fluid and refill it with non-chlorinated water, add ten wheat seeds, cover the top with foil, label, and return to the storage area.

Let the five-gallon bottle stand for two weeks to permit population levels to increase.
After two weeks, use a stereomicroscope to check the number of *Chilomonas* present (See Checking Population Levels, p. 16). (The concentrations should be high after two weeks.)

> **Problem**: If the numbers of *Chilomonas* appears low, add another flask of *Chilomonas*. Examine the liquid under a stereomicroscope after an additional week. It is best to begin the whole process again using clean equipment if the population numbers are still low.

Preparing a stock culture of *Paramecium*

Materials needed
One-liter flask of *Chilomonas*
Two-liter flask of *Paramecium*
Five-gallon glass bottle
Autoclaved wheat seeds
Non-chlorinated water
Aluminum foil
Long-handled scrub brush
Plastic funnel
Hot water [>70°C (>170°F)]
Labeling tape
Sharpie pen

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Procedure:

To start a *Paramecium* stock culture, you need a clean five-gallon bottle (See Culturing *Chilomonas*, p. 12).

Place a clean funnel into the top of the five-gallon bottle.

Add approximately 2.5 liters of non-chlorinated water to the bottle.

Pour all but 50 ml of a one-liter flask of *Chilomonas* culture into the funnel.

IMPORTANT! Do not use the entire contents of a flask of *Chilomonas* or *Paramecium*. Always save some of the liquid in the flask to restart the culture.
Pour most of the contents of a two-liter flask of *Paramecium* culture into the funnel.

Cover the top of the bottle with aluminum foil.

Label the bottle to show the date and contents.

Take the two flasks, each still containing about 50 ml of fluid, and refill with non-chlorinated water. Add ten wheat seeds to each flask. Cover the tops with foil, label with the date, and return the flasks to the storage area.

Let the five-gallon bottle stand for two weeks to permit population levels to increase.

After two weeks, check the concentration of *Chilomonas* and *Paramecium* present by using a stereomicroscope (See Checking Population Levels, p. 16). (The concentrations of both should be high after two weeks.)

> **Problem:** If the numbers of either/both *Chilomonas* or *Paramecium* appear low, add another flask of the needed animal. After one more week, examine the liquid under a stereomicroscope. If numbers are still low, it is best to begin the whole process again using clean equipment.

Preparing a stock culture of cyclops

A stock culture of cyclops in a single five-gallon bottle will provide enough cyclops to seed a large number of trays. For this reason, it may not be necessary to maintain more than one bottle of each cyclops species. A bottle of cyclops may be obtained by adding <20 cyclops to a bottle containing a culture of *Paramecium*.

Materials needed
Five-gallon glass bottle of *Paramecium* stock culture
Non-chlorinated water
Aluminum foil
Sterile pipette
Labeling tape
Sharpie pen

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Procedure:

Start with a *Paramecium* stock culture that has high populations of both *Chilomonas* and *Paramecium*. (See Checking Population Levels, p. 16).

Use a pipette to add <20 of the desired species of cyclops to the *Paramecium* stock culture.

> **IMPORTANT!** Do not introduce more than 20. Introducing a larger number of cyclops will lead to an excessive number of juveniles and deplete the food supply. The net yield of adult cyclops will be greatly reduced.

Cover bottle top with aluminum foil.

Temperature and lighting needs are flexible. Just keep the bottle at normal room temperature where it receives typical room lighting.

Label the bottle to show the date and the cyclops species in the bottle.

The number of adult cyclops in the bottle should reach a peak within three weeks.

MAINTAINING A CYCLOPS STOCK CULTURE

A five-gallon bottle containing both *Chilomonas* (food for young cyclops) and *Paramecium* (food for older cyclops) permits the number of cyclops to expand until they reach a peak in three weeks. This is the best time to use a stock culture to start production trays or additional bottles.

After the cyclops reach their peak, they will deplete their food supply, and their population will decline unless supplemental food is added to the stock culture bottle. High cyclops levels can be maintained for an additional two months or more by adding newly emerged brine shrimp (See Production Suppliers, Appendix III).

Production of Brine Shrimp using a hatchery

Materials needed

Brine shrimp cysts ("eggs")
Non-iodized salt
Shrimp hatching container w/ an aeration pump
Electric light
Measuring tablespoon
Plastic funnel
2 two-liter containers
One-liter container
Hand net with 200 um netting
Non-chlorinated water
Tap water
Pipette

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Procedure:

(You can use the following procedure or you can choose to follow the rearing instructions which are printed on the package of brine shrimp cysts. Both procedures yield excellent results. However, if large scale production of cyclops is planned, constructing or purchasing a hatchery such as the one shown in the diagram will save time and energy.)

NOTE: Brine shrimp form a protective covering (cyst) when faced with stressful conditions. In their encysted form, brine shrimp are able to survive for years in the laboratory when refrigerated. In nature and in the hatchery, the brine shrimp emerge from their cysts when environmental conditions become favorable for growth and reproduction.

To produce brine shrimp:

Make sure the drain of the shrimp hatchery is closed.

Fill the shrimp hatchery with two-liters of non-chlorinated water.

Turn on the aeration pump.

Add two tablespoons of non-iodized salt to the water.

Allow 30 minutes for the salt to dissolve in the water and for

the water to warm to room temperature

Remove a packet of brine shrimp cysts from the refrigerator. Add one tablespoon of brine shrimp cysts to the water. maintain the room temperature at about 30°C (86°F).

NOTE: Hatch should be complete after approximately 30 hours.

CAUTION! 24 hours may not be long enough for a complete hatch, but by 48 hours the newly emerged shrimp (lacking both food and the stored energy necessary to again encyst) will have died.

To remove brine shrimp from the hatchery:

Stop the aeration pump.

Turn on the electric light at the bottom of the hatchery.

Wait 30 minutes. (Egg shells will float to the surface and shrimp will be drawn down to the light.)

Place a two-liter container under the drain spout of the hatchery.

Drain as much liquid as possible without draining shells.

Place this shrimp-containing liquid to the side and immediately clean the hatchery:

Place another container under the hatchery drain to complete the draining of the hatchery.

Drain the water still remaining in the hatchery into this container.

Pour the water containing the egg shells down the sink drain.

Place the emptied container under the drain once more.

Flush the hatchery with tap water and pour this water down the sink drain.

Close the drain valve.

Remove brine shrimp from the hatching water by pouring the water containing the brine shrimp through a 200 um mesh hand net or filter cloth.

IMPORTANT! Very salty shrimp will remain in the net after the water has drained. This salt needs to be removed before the shrimp to prevent harm to fresh-water cyclops and *Paramecium.*

Flush the salt from the shrimp by slowly running at least threeliters of non-chlorinated water through the net. (Be careful to prevent shrimp from being washed over the sides of the net.)

Use the running water to concentrate the shrimp into a corner of the net.

Hold the net above a one-liter container half-full of non-chlorinated water and slowly invert the net.

Place the section of the net that contains the concentrated shrimp into the water and move it laterally back and forth to flush the shrimp off the net and into the container.

To feed shrimp to cyclops, use a pipette to draw out about ten ml of well-stirred, shrimp-filled water. Add this liquid to the five-gallon bottle containing the cyclops stock culture.

(One brine shrimp hatching will provide enough supplemental food for 4 five-gallon bottles of cyclops stock culture, plus four production trays.)

Repeat the entire procedure every four days for as long as cyclops cultures in bottles or trays need to be maintained.

A large cyclops population can be maintained for about two months with this procedure.

CLEANING A PRODUCTION TRAY

The production trays used in our laboratory are 4' x 8'. These trays are filled with water to an average depth of about 2". Trays or pans of other sizes can be used for cyclops production. However, a tray with a high ratio of surface area to water volume will produce the maximum numbers of cyclops. A frame holding a five tray stack (as shown in the illustration) results in 160 sq. ft. of surface area within a floor space of only 32 sq. ft. This production set-up will provide approximately 100,000-150,000 adult cyclops every two months.

NOTE: The following instructions apply specifically to our production system. However, the procedures described will have general application to any successful production system.

Materials needed
Production tray
Two garden hoses
Household bleach
Soft cloth or nylon bristle brush
Water drain (preferably a floor drain)
Tap water

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Procedure:

Close drain valve on tray.

Fill the production tray nearly to the top with tap water.

Gently scrub the tray with a soft cloth or nylon bristle brush.

Obtain a hose long enough to reach easily from the tray's drain valve to the floor drain and attach one end to the tray's drain valve.

Insert the other end of the hose into the floor drain.

Open the tray drain valve and allow water to drain out.

Use a hose to wash off the sides of the tray with tap water.

Close the tray drain valve.

Completely fill the tray with tap water.

Add 200 ml of bleach.

Leave the bleach-water mixture for one hr.

After that time has passed, gently scrub the tray with a nylon brush to loosen any dirt.

Open the drain valve.

Rinse the tray thoroughly using non-chlorinated water.

Close the drain valve.

Remove the hose attached to drain valve and put the hose away.

Let the tray dry for at least 24 hours before use.

SETTING UP A PRODUCTION TRAY

> **NOTE:** The following instructions apply specifically to our production system. However, the procedures will have general application to any successful production system.

Materials needed

Production tray with a high surface area to water volume ratio
Non-chlorinated water
Triple beam scale (or other balance)
Autoclaved wheat seeds
Five-gallon bottle of *Chilomonas* stock culture
Five-gallon bottle of *Paramecium* stock culture
Labeling tape
Sharpie pen
Stereomicroscope
Cyclops of the desired species

Procedure:

Follow the recommended cleaning procedures if the tray was not properly cleaned after its last use (See Cleaning a Production Tray, p. 26). If the tray was cleaned, but it was more than a month ago, it needs to be rinsed with hot water before being used for cyclops production.

Make certain the tray's drain valve is closed.

Add non-chlorinated water to the tray until all the bottom is covered by at least four cm of water.

Let the water stand until it reaches room temperature (about three hours).

Use the triple beam scale to measure out 60 gm. of wheat seeds.

Scatter these wheat seeds over the water surface of the tray.

Add the contents of a five-gallon bottle of *Chilomonas* stock culture to the tray (See Culturing *Chilomonas*, p. 12).

Attach a strip of labeling tape on the edge of the production tray. Record the date and the tray's contents using a Sharpie marker.

If the bottle does not contain too much debris, restart a *Chilomonas* stock culture in the same bottle. More than two cm of debris over the entire bottom of the bottle may require starting a stock culture in a new bottle (See Preparing a Stock Culture, p. 17).

If the bottle does not contain too much debris, add 70 wheat seeds, and charcoal-filtered water.

Label the bottle and return it to the shelf.

Let the tray stand for three days to permit the *Chilomonas* to multiply.

Add the contents of a five-gallon bottle of *Paramecium* stock culture (See Culturing *Paramecium*, p. 14).

Note the addition of *Paramecium* on the tray's label.

Restart a *Paramecium* culture in the same bottle if it does not contain too much debris. Otherwise, start a culture in a new bottle.

Maintain the tray at about 30°C (86°F). Any light/dark cycle or even total darkness is acceptable for *Chilomonas* and *Paramecium* multiplication. However, prolonged bright lighting will encourage the growth of algae. Algal growth will remove nutrients from the water and may result in decreased cyclops production.

After two weeks, check to see if the populations of *Chilomonas* and *Paramecium* in the tray are high (see Checking Population Levels, p. 16). If so, proceed to the next step. If not, check again after one week.

Once *Chilomonas* and *Paramecium* populations are high, introduce approximately 100 adult *Mesocyclops* or approximately 300 *Macrocyclops* from a stock culture to the tray.

> **IMPORTANT!** Do not introduce more than 100 Mesocyclops or 300 Macrocyclops to a tray. Introducing a larger number of cyclops will lead to an excessive number of juveniles, which will deplete the food supply. The net yield of adult cyclops will be greatly reduced.

Fewer Mesocyclops are used because *Mesocyclops* reproduces more rapidly than *Macrocyclops.* The cyclops will reach peak numbers within 3 to 4 weeks.

COMMON PROBLEMS AND SIMPLE SOLUTIONS

PROBLEM I: White film forms on water surface.
SOLUTION: Unsightly, but does not seem to affect cyclops production. Can be skimmed off with a hand net.

PROBLEM II: Black "worms" appear in water.
SOLUTION: These are "sewer fly" larvae. The adults are small, black, and moth-like. The larvae can be eliminated through an application of *Bacillus thuringiensis* var. *Israelensis* (B.T.I.) at label recommended levels without harming the cyclops or their food.

MAINTAINING A PRODUCTION TRAY

It is most energy and cost efficient to time cyclops production to achieve the maximum number of cyclops at exactly the time they are needed for mosquito control. However, once a tray reaches maximum population (maturity) it can be maintained through supplemental feeding. The supplemental foods we use are brine shrimp and brewer's yeast. Cyclops can be maintained at high concentrations for at least two months with the addition of these supplemental foods.

Brine Shrimp

The same procedure and time-line described to maintain a five-gallon bottle of cyclops are used to hatch brine shrimp for a production tray (See Maintaining a Cyclops Stock Culture, p. 22). The only change from the stock culture guidelines is that the amount of brine shrimp necessary for maintaining a production tray is proportionally larger. Add 1/4 of a brine shrimp hatch to a production tray every four days.

Brewer's Yeast

Materials needed
Brewer's yeast
Weighing paper
Electronic balance
One-liter container
Non-chlorinated water

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Procedure:

Place a weighing paper on the pan of an electronic balance (or other accurate balance).

Set the balance to read 0.00

Gently spoon yeast onto weighing paper until the scale reads five gm.

Turn off the balance and pour the five gm. of yeast into about 0.5 liters of non-chlorinated water.

Stir the water until the yeast dissolves.

Pour the yeast-containing water into the production tray.

Repeat this procedure every other week until the cyclops are harvested for use.

HARVESTING THE CYCLOPS

When cyclops are removed from production trays, they must be concentrated and separated from the wheat seed debris in the tray water. The procedure is time consuming because debris clogs the netting that is used to remove cyclops from the water. Nonetheless, taking the time to do the separation properly if important because: 1) concentrating the cyclops in debris-filled water will result in oxygen depletion and 2) concentrating the cyclops in water that is debris-free will facilitate their subsequent handling and use.

Materials needed

Hose
Water drain (floor drain is preferable)
Two metal strainers
Netting with 200 um mesh
Non-chlorinated water
0.5-gallon plastic food containers with lids
Five-gallon bucket

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Procedure:

Attach hose to drain valve of tray, and extend the other end of the hose to the sink or floor drain.

Line a metal strainer with 200 um mesh netting. (A 200 um mesh will capture adult and subadult cyclops. A 80 um mesh needs to be used if you wish to capture nauplii.)

Place a second metal strainer into the first one (like stacked spoons). The netting must be held between the strainers, essentially making a sandwich with the netting as the "meat".

Place the nozzle of the hose into the "net sandwich" so that draining water flows through the strainers and net and into a sink or floor drain.

Open the drain valve on the tray.

As the water from the tray drains, use a stream of non-chlorinated water to wash cyclops and debris towards the drain of the tray.

NOTE: The non-chlorinated water can be poured from containers of dechlorinated water or provided from a hose attached to a charcoal filter.

PROBLEMS: If the strainer appears close to overflowing, partially close the drain valve. If the strainer continues to nearly overflow, completely close the valve and remove cyclops and debris from the net before draining the rest of the tray. Do not allow the net to overflow. If you do you will lose many of the cyclops.While the tray drains, continue using a stream of non-chlorinated water to wash all contents of the tray into the drain. (Concentrate the stream onto the sides of the tray. Cyclops will often strand themselves rather than be carried along by a current.)

If the net becomes clogged, close the tray's drain valve.

Fill a one-gallon bucket with non-chlorinated water.

Lift the top strainer away from the net and the other strainer beneath it. (The top strainer should have trapped most of the wheat seed debris and unfortunately many cyclops.)

Empty the contents of the top strainer into the water-filled bucket.

Place the upper strainer back on top of the net and other strainer.

Open the valve, and continue passing water through the net sandwich of strainers.

After the draining is complete, close the drain valve.

Fill the tray with non-chlorinated water.

Empty the top strainer into the gallon bucket.

Lift the net from the lower strainer. (The net should contain most of the cyclops.)

Use a gentle flow of non-chlorinated water to concentrate the cyclops into one section of the netting.

Invert the netting into a 0.5-gallon plastic food container that is half-full of non-chlorinated water.

Move the netting laterally back and forth in the water to remove the cyclops.

After rinsing the metal strainers and mesh, again make the strainer "sandwich".

Pour the bucket of debris obtained from emptying the top strainer into the tray.

Drain this material using the same procedure outlined above.

Discard any material collected in the top strainer.

Rinse the cyclops containing material from the mesh.

Once a tray has been drained, it should be cleaned before any remaining undrained debris has time to dry and harden (See Cleaning a Production Tray, p. 26).

You now have a water-filled container holding a very high concentration of cyclops and debris (primarily fragments of rotting wheat seed). This mix of cyclops and debris is acceptable for application to swales if used within a day. However, if any type of hand or broadcast sprayer is to be used, the cyclops must be separated from the remaining debris before application to tires or other containers. Debris will clog sprayer nozzles if not removed.

Separating cyclops from debris

Procedure:

Fill a five-gallon bucket nearly to the top with non-chlorinated water.

Pour the contents of the plastic container (water, debris, and cyclops) into this bucket.

Allow approximately ten minutes for the debris to settle.

Pour the water from the bucket through a 200 um net to collect the cyclops. Pour the water rapidly to prevent cyclops escape but stop pouring before debris-containing water starts to pour out of the bucket.
Fill the bucket again with non-chlorinated water. Wait for the debris to settle. Pour the water rapidly from the bucket into the net. Once again

stop pouring before the debris-containing water starts to pour out of the bucket.

Repeat the process up to five times to separate as many cyclops as possible from the debris.

After each draining, invert the net into a 0.5-gallon plastic container half-full of non-chlorinated water. Move the net in the water so as to free all cyclops from the net.

Pour the remaining contents of the bucket into the floor drain after the fifth draining has been completed.

The cyclops in the 0.5 gallon plastic container can now be divided into approximately equal smaller groups and placed into containers for use in mosquito control.

FIELD APPLICATION OF CYCLOPS

Application to tires

The New Orleans Mosquito Control Board uses cyclops to control *Aedes albopictus*, *Aedes aegypti*, and *Aedes triseriatus* larvae in tires and other containers. *Mesocyclops longisetus* is the best New Orleans cyclops species for tires because it tolerates the high water temperatures that occur in New Orleans during the summer. [*Mesocyclops longisetus* survives temperatures up to 43°C (110°F)]. *M. longisetus* usually survives in tires for years, but it is killed when tires dry out completely. It can also be killed by unusually cold weather during the winter. *M. longisetus* cannot survive prolonged water temperatures below 2°C (35°F). For this reason, south Louisiana represents the northern edge of its known habitation range. It is found throughout much of Central and South America. Containers that have been treated with *M. longisetus* should be checked every spring to verify that *M. longisetus* is still there. Introduce new cyclops to a container whenever cyclops are not seen immediately. Another U.S. species, *Macrocyclops albidus*, has also proven to be an effective predator of mosquito larvae. It is much less tolerant of heat, but will survive cold weather as long as some water remains unfrozen. It maintains thriving populations in containers and is common in many natural water bodies (large and small) throughout the United States and into Canada.

Using a backpack sprayer for cyclops distribution

We usually employ a Swissmex SVV Survivor backpack sprayer

(two-gallon capacity) to transport cyclops to the field and to apply them to tires and other containers.

Materials needed
Lidded containers, holding a concentration of cyclops
Two-gallon backpack sprayer
Non-chlorinated water
B.T.I. (If mosquito larvae need to be controlled immediately)

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Procedure:

Remove the nozzle from the sprayer and keep the nozzle off while using the sprayer to distribute cyclops.

Remove the cover from the tank of the sprayer and pour in one gallon of non-chlorinated water.

Add approximately 10,000 cyclops which have been concentrated and completely separated from any wheat seeds or debris.

Fill the tank with non-chlorinated water to the two gallon mark.

If there are already mosquito larvae in the tires to be treated, add liquid *Bacillus thuringiensis* var. *Israelensis* (B.T.I.) concentrate to the tank. Follow label instructions for the proper concentration of B.T.I. (B.T.I. has no adverse effect on cyclops at the concentration used for mosquito control.)

Replace the tank cover and pressurize the tank with the plunger. (The pressure necessary for application has no adverse effect on cyclops.)

Take the tank to the field for cyclops application.

At the application site, squirt one spray (about 30 ml) of the tank's contents into each container.

> A two gallon tank will treat about 250-300 tires or other containers. About 40 cyclops will be introduced into each container. (A single female introduced into any container will potentially produce enough offspring in several generations to control mosquito larvae. For this reason, depositing fewer cyclops into each container will also result in the same

control effect after several months.)

Using a broadcast sprayer for cyclops distribution to tires

Another way to apply cyclops to a pile of tires is to broadcast the cyclops over the pile with an "air-blast" sprayer such as a Buffalo Turbine or "Scorpion". The sprayer adds the cyclops to an a fast-moving stream of air. Cyclops are hardy animals but they usually do not survive passing through a high-pressure nozzle.

Materials needed

A concentration of cyclops in lidded 0.5-gallon plastic containers
Five-gallon bottle or bottles
Non-chlorinated water
Truck-mounted broadcast sprayer

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Procedures:

Take the cyclops to the spray site in 0.5-gallon plastic containers (approx. 20,000 cyclops/container).

Immediately before spraying, pour the cyclops from a plastic container into a five-gallon bottle already nearly filled with non-chlorinated water.

> **NOTE:** Cyclops will concentrate at the bottom of the five gallon bottle if cyclops are added to the bottle too soon before spraying takes place. If the cyclops are allowed to concentrate in the bottom of the container they will fail to be distributed evenly over the treatment area.

Place the intake tube of the sprayer into the five-gallon bottle.

Spray the five gallons of water (containing cyclops) over about 500 tires. This should assure total coverage of all tires in the top three layers of tires. Research conducted by NOMCB has demonstrated that mosquito production in tire piles takes place primarily in the top three layers. Tires in these top three layers will be expected to receive an average of about 12 adult cyclops per tire.

Repeat the procedure until all tires in the pile are treated.

Application to Swales

The time between November and April is the main season for swale (temporary water bodies) mosquito production in New Orleans. *Macrocyclops albidus* is the best species to apply to swales because it survives cold winter temperatures better than other species of larvarvorous cyclops. *Macrocyclops albidus* should be introduced only to swales that are expected to retain at least a pocket of water or moist soil through the winter since it will be killed if a swale dries completely.

Cyclops do not eliminate mosquito larvae from swales as effectively as they eliminate mosquito larvae from tires. Field trials in New Orleans have shown that production of *Culiseta inornata*, *Aedes vexans*, and *Aedes sollicitans* is substantially reduced by the introduction of *Macrocyclops albidus*. *Macrocyclops albidus* does not reduce *Culex salinarius* and some other species of *Culex* sufficiently to be of use for controlling these species.

Materials needed
Lidded plastic containers or jars (at least 200 ml capacity)

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Procedure:

NOTE: It is better to put cyclops into plastic containers at the production facility before transporting them to the field. Laboratory lighting conditions are better for seeing the cyclops well enough to allocate the correct number for introduction to each swale.

Fill each container with about 100 ml of non-chlorinated water.

Place about 500 cyclops into each container.

Take the containers to appropriate swale sites.

Pour the contents of at least one container into each swale.

NOTE: The contents of one container are sufficient to treat about 1/4 acre (1,000 square meters). If a swale is large, it is advisable to introduce the contents of several containers at several different parts of the swale.)

SHIPPING CYCLOPS

Occasionally, cyclops must to be shipped to another facility. When cyclops are stored together in small containers with limited food, they will feed on one another. This obviously presents a problem when shipping. We have partially solved this problem by storing cyclops on a damp sponge. Placing the cyclops on a damp sponge prevents desiccation but does not provide enough water for them to move about and eat one another. Each square centimeter of sponge provides a holding facility for up to 20 cyclops.

Materials needed

Plastic container with a tight-fitting lid
Polyester sponge
Pipette

Procedure:

Cut a polyester sponge into a shape that will fit snugly into a plastic container with a tight fitting lid.

Submerse the sponge in non-chlorinated water.

Remove sponge from water and hold it in your hand.

Pour cyclops-containing water slowly over the top of the sponge, distributing the cyclops over the entire surface. (Pour on no more than 20 cyclops per square centimeter of sponge.)

Place the sponge into the container.

Place the lid onto the container.