

ZIRC Paramecia Procedure

List of Ingredients and Materials:

Cultures

- Sterile containers or petri-dishes, at least 150x10mm (large surface area is ideal)
- Autoclaved fish water or deionized water
- Nutritional brewer's yeast tablets (crushed and/or powdered)
- Autoclaved dry whole wheat kernels

Sub-cultures

- 500 to 2000ml plastic containers with large surface area
- Dechlorinated filtered tap water and/or filtered fish system water
- Nutritional brewer's yeast (crushed and/or powdered)
- Autoclaved dry whole-wheat kernels

Other Necessary Items

- Measuring spoons, 0.05 and 0.1 gram sizes
- Measuring spoons, ¼ tsp. and ½ tsp.
- Warm room with medium light (70-80°F)
- Strainers/sieves made from 105 and 20 micron polyester filter cloth (figure 2)
- Paramecia collector (for description, see figures 3-4)

List of terms:

Cultures-

Stage 1 of the paramecia production process. Sterile paramecia food cultures grown in **sterile** containers or large petri-dishes (150x10mm) using **sterile** fish water or deionized water. These are used to make new cultures and sub-cultures.

Sub-cultures-

Stage 2 of the paramecia production process. Clean, but not sterile, paramecia grown in 500 to 2000mls **dechlorinated filtered tap or fish system water**. Sub-cultures are inoculated using the cultures. The paramecia grown in sub-cultures is fed to the larval fish.

Additional Information:

- Sub-culture containers should hold 500 to 2000mls water, have a large surface area, be easy to wash, and ideally withstand high temperatures so that they can be cleaned using a cage washer or high temperature dishwasher. The large water surface to air ratio is vital in growing dense paramecia cultures and sub-cultures.
- Both cultures and sub-cultures will require approximately 2 weeks to reach optimal density.

Making Cultures

Obtaining a Starter Culture

When culturing paramecia you are essentially creating an ecology in which microorganisms thrive. There are a host of other organisms besides paramecia that thrive in the same conditions, so it is important to monitor your cultures to make sure you haven't introduced any unwanted organism(s).

Starter cultures at ZIRC are routinely monitored for the presence of opportunistic organisms. ZIRC has on occasion seen a small percentage of vorticella rotifers in our colony. Rotifers are a known food source for zebrafish. While the rotifers have not had any spurious effect on the paramecia cultures, we have performed serial dilutions on the ZIRC starter cultures to reduce the rotifers to undetectable levels. Occasionally doing a serial dilution on established cultures in your facility is a good way to ensure the cleanliness of your colony and can be done at anytime if contamination occurs.

If you have questions regarding anything you see in your culture or observe in your colony, please feel free to contact us.

Preparation

Before working with paramecia it is a good idea to start with a sterilized work surface, which will reduce the chance of contaminating the cultures.

First, a small amount of autoclaved dry whole-wheat kernels (1tbs) are boiled in autoclaved water (autoclaved fish or deionized water, depending on what is available to you) for 10 to 15 minutes. While the kernels are boiling, set out five to ten large sterile containers or petri-dishes at least 150x100mm in size. Fill each dish half full with sterile water. To each dish also add approximately 0.01 grams of powdered brewer's yeast. When the wheat is done boiling, remove it from the hot plate, pour off any excess liquid and add 4 wheat kernels to each plate. Wait a couple of minutes to allow the wheat to completely cool off in the water before starting the next step.

Now you are ready to inoculate each petri-dish using the paramecia starter culture. The paramecia starter culture obtained from ZIRC should be divided up equally between the five to ten petri-dishes you have prepared. If you are not using ZIRC cultures, then add approximately 15 to 30ml of dense culture to each dish. Cover each petri-dish and label it with the date that it was made. The ideal temperature for growing paramecia cultures is between 75 to 80 degrees Fahrenheit. To reach optimal density, allow the cultures to reproduce and grow for approximately 2 weeks.

Refreshing Cultures

Cultures need to be refreshed every 7 to 14 days to prevent die off. Generally, two sets of cultures are kept going at all times. The older set should be used to make a new set of cultures and sub-cultures.

Take the oldest set of cultures, which should be 7 to 14 days old, and check each dish under a microscope for density and possible contamination. Choose the densest

culture and divide it equally to the new set of cultures/petri-dishes. The remaining culture plates should be checked for contamination and density and then set aside for inoculation of the sub-cultures. It is always a good idea to keep one or two of the oldest cultures around until the following week in case you need a back-up.

Making Sub-cultures

Preparation

Set out the required number of plastic containers (see figure 1). For example, if you are making sub-cultures on Monday, calculate how much paramecia you will need in approximately 2 weeks for feeding larval fish and then set out the appropriate number of containers. One petri-dish of culture can be divided to make 1 to 3 sub-culture containers.

Fill each container with 500 to 2000ml of dechlorinated or filtered water (tap or fish water). Add 0.05 to 0.10g powdered brewer's yeast tablets depending on the volume of the container. Also add $\frac{1}{4}$ to $\frac{1}{2}$ tsp dry whole-wheat kernels that have been autoclaved. Each container then receives 30 to 50mls paramecia culture, thus creating the sub-cultures. Be sure to stir and mix the cultures before adding them to the sub-culture containers. Cover and label the sub-cultures with the date.

The sub-cultures take approximately 2 weeks to reach optimum density and are usable for up to three weeks. It is recommended that all stages of the paramecia process be monitored to insure that the cultures and sub-cultures are contaminant-free and healthy.

Harvesting Paramecia from Sub-Cultures for Larval Feeding

Calculating the Amount of Concentrated Paramecia Needed

Larval zebrafish (4-8 dpf) are fed concentrated paramecia twice per day. To calculate the amount of concentrated paramecia needed for both the morning and afternoon feedings, start by counting the containers or tanks of larval fish that are 4-8 days post fertilization. The amount of paramecia that each sub-culture will make varies depending on several factors including container volume, environmental conditions such as temperature and light intensity, and the age of the paramecia cage. At ZIRC, each 1000ml container of paramecia makes 300-500mls of concentrated paramecia.

The best way to determine how much food each cage will make in your facility is to prepare one cage to the proper food density. After seeing how much food each cage makes, you can then use that as a guide to determine your daily food needs. The proper density for concentrated paramecia, as prepared for feeding fish, should be 100 paramecia/ml fish water.

Concentrated Paramecia Preparation

Paramecia needs to be thoroughly filtered and rinsed before they can be concentrated and fed to the nursery. Pour each container of sub-culture through the 105 micron strainer (See figure 2), collecting the liquid and paramecia in a container below and discarding the debris caught in the strainer. Rinsing the strainer between cages will make this process quicker and easier.

Pour the filtered paramecia into the paramecia collector (See figures 3-4), making sure that both valves are shut, and top off with fish water. Let the collector sit for 15 to 20 minutes. This will allow the paramecia to gather at the top of the collector. To collect the paramecia, put a large container under the top valve. Open the valve and allow all of the paramecia off the top of the collector to drain into the container. Shut the top valve and refill the collector with fish water. Repeat this step until you have retrieved the majority of the paramecia. This usually takes 2-3 times. After the last collection, the collector can be emptied and the remaining liquid discarded by opening the bottom valve and allowing it to drain.

The last step in the filtration process is to remove any residual ammonia in the water from the paramecia and replace it with ammonia-free fish water. This process is done by pouring the previously collected paramecia into a 20 micron strainer/sieve (see figure 2). The paramecia are too large to fit through the 20 micron filtered material. Unlike the 105 micron strainer, the 20 micron strainer retains the paramecia in the strainer and discards the liquid. After the majority of the water is drained, use fresh fish water to cleanse the paramecia of any remaining ammonia.

After rinsing, pour the paramecia into a clean container. Fill the container with clean fish water until a concentration of 100 to 150 paramecia/1ml fish water is achieved and feed. Note: Before feeding the paramecia, check the ammonia level with a test kit. If it shows any trace of ammonia, repeat the 20µm mesh straining process. To prevent the mesh strainer from clogging with debris, rinse using a high pressure spray.

Materials and Figures: Sub-culture Containers



Figure 1. Examples of sub-culture containers ranging in size from 500 to 2000ml. The container on the left is a polycarbonate Nalgene animal cage (24x14x13cm). It has a volume of approximately 2 liters. The container on the right is a Thoran crossing cage and holds approximately 1 liter.

Micron Mesh Strainers/Sieves

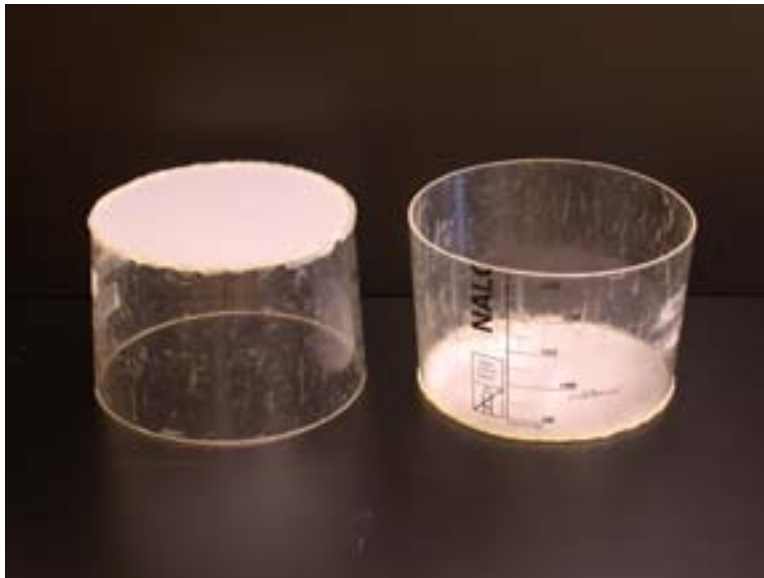


Figure 2. Strainers used in the cleaning and concentrating of paramecia. The strainers are hand made by taking a large plastic beaker and cutting it into cylinders. Polyester filter or bolting cloth is then fixed to one side using hot glue.

Paramecia Collector



Figure 3. Paramecia collector. The paramecia collect at the top of the carboy and are then removed by the pipe fittings near the top. The collector is made from a carboy with a spigot at the bottom and a modified suction drain (see figure 4). A four gallon carboy is recommended for larger facilities, while a 2 gallon carboy will work well for those needing only small amounts of paramecia.

Pipe Fittings for the Paramecia Collector

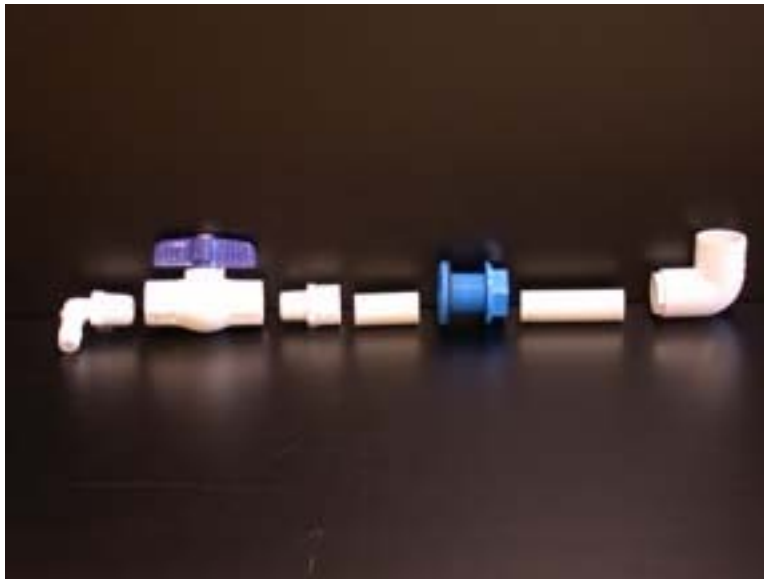


Figure 4. Paramecia suction drain. The suction top drain is made using (from left to right): 90° tubing adapter (1/2" id, 1/2" threaded), 1/2" PVC ball valve, 1/2" male adapter, a 2" length of 1/2" PVC pipe, 1/2" bulk head fitting, 1/2" PVC pipe, 1/2" 90° elbow. The length of the second piece of PVC pipe is determined by the type and size of container. The elbow needs to sit directly below the center of the opening in the carboy. Cut the PVC pipe to accommodate this. We recommend press fitting the pieces together as opposed to PVC gluing them. This makes dismantling easier when the unit needs cleaning.