ZIRC Paramecia Instructions

List of Ingredients and Other Necessary Items:

Cultures

- Sterile petri-dishes, at least 150x10mm
- Fish water or dechlorinated tap water, Autoclaved
- Nutritional brewers yeast tablets, any brand
- Autoclaved whole wheat kernels

Sub-Cultures

- Plastic containers, 500-1000mls (figure 1)
- Powdered brewers yeast, any brand
- Autoclaved whole wheat kernels
- Dechlorinated tap water or fish water, filtered

Cages

- 500-1000ml plastic containers
- Powdered brewers yeast
- Autoclaved whole wheat kernels
- Dechlorinated tap water or fish water, filtered

Other Necessary Items

- Measuring spoons, .05 and .1 gram sizes
- Warm room with medium light (70-80°F)
- Strainers made from 105 and 23 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 at least 150x10mm glass or plastic petri-dishes, used to make paramecia

Sub-cultures-

Stage 2 of the paramecia production process. Clean, but not sterile, paramecia cultures grown in 500-1000mls filtered fish water or dechlorinated tap water. These are used to make *Cages.*

Cages-

Stage 3 of the paramecia production process. Clean, but not sterile, paramecia grown in 500-1000mls filtered fish water. The only difference between *Cages* and *Subcultures* are that in cages, the paramecia population is started with sub-culture and in the sub-cultures the paramecia population is started with cultures. This is done to achieve optimum population density when harvesting paramecia for use in the nursery and to produce enough culture to inoculate a large number of cages.

The container used for cages and sub-cultures should hold 500-1000mls water and be easy to wash, and ideally withstand high temperatures so that they can be cleaned using a cage washer or high temperature dishwasher. The containers should also allow for a good water surface to air ratio, which is vital in growing quality paramecia.

Making Cultures

Obtaining a Starter Culture

Starter cultures can be obtained from ZIRC or other commercial retailers such as Carolina Biological. Commercial cultures almost always contain other organisms that should be removed before using them in your facility. Cultures obtained from ZIRC have already been "cleaned up" but it is still a good idea to carefully examine each culture under a microscope before using them.

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 whole-wheat kernels (1tbs) are boiled in autoclaved water (autoclaved fish or dechlorinated tap water, depending on what is available to you) for 10-15 minutes. While the kernels are boiling, set out seven large petri-dishes at least 150x10mm in size. Fill each dish half full of autoclaved water. To each dish also add one small piece of brewer's yeast tablet. The pieces are approximately .01 grams each and are about 2x2x2mm in size. When the wheat is done boiling, remove it from the hot plate, pour off excess liquid and add 4-5 of the wheat kernels to each plate. Wait a couple of minutes to allow the wheat to cool off completely in the water before starting the next step.

Now you are ready to add the paramecia starter culture. The paramecia starter culture obtained from ZIRC should be divided up equally between the seven plates you have prepared. If you are not using ZIRC cultures than add approximately 15ml of dense culture to each petri dish. Cover each petri-dish and label it with the date that it was made. The ideal temperature for growing paramecia cultures is between 75-80 degrees Fahrenheit.

Refreshing Cultures

Culture sets (each set of seven cultures made from one petri-dish) need to be refreshed every 7 to 14 days. Generally, two sets of cultures are kept going at all times so the older set should be used to make a new set of cultures and the subcultures.

Take the older set of cultures, which should be 7-14 days old and check each dish

under a microscope to check for density and any possible contamination. Choose the most dense culture and use this to make the new set of cultures. Pick out the 2nd and 3rd best cultures and set these aside to make subcultures. The remaining culture plates should be checked to make sure they are healthy and kept until the next week's production day as back up.

Making Subcultures

Preparation

To make subcultures, set out 4 clean cages and fill each one with 500-1000ml of either sterile or filtered fish water, or filtered de-chlorinated tap water.

For smaller facilities, which don't require a large amount of water for paramecia production, autoclaving is the easiest way to get clean water for paramecia production.

For larger facilities, that will need more food and there for more water, a filtration unit is probably more appropriate. At ZIRC, all the paramecia water is filtered through a .23um Millipore Stainless-Steel Pressure Filter.

To each cage, add .05g powdered brewer's yeast tablets. Powdered brewer's yeast products are available but we have found that they don't work as well as the powdered tablets. Also add 10 dry whole-wheat kernels that have been autoclaved. Ten kernels is approximately 1/4 tsp. Retrieve the 2 culture dishes that were set aside and add 1/2 of each culture to each cage.

Label the cage with the date, mark it as a subculture, and cover the containers with a clear lid (long pieces of plexiglass work well). Do not cover the containers with airtight lids! If container lids are tight fitting, drill some air holes in them.

The subcultures generally take 7-10 days to reach optimum density but will remain at that density for about a week and usually up to 2 weeks.

Making Cages

Preparation

Set out how many cages you would need for food the following week. For example, if you are making food on Monday, calculate how much food you will need for the week starting on the following week based on how many fish will be in the nursery. This accounts for the amount of time the paramecia need to repopulate and when they will be ready for harvest.

Fill each cage with 500-1000ml of the same water used to make subcultures. To each cage, add .05g powdered brewer's yeast tablets. Also add 1/4tsp dry whole-wheat kernels that have been autoclaved. Each cage receives 30-50mls subculture. Be sure to stir the subculture before adding it to the cages because the paramecia will collect in pockets within the cage. Label the cages with the date and cover.

The cages usually take approximately 7-10 days to reach optimum density and are usable for up to two weeks after. It is recommended that all stages of the paramecia are checked regularly to make sure they are contaminant free and healthy.

Harvesting Paramecia from Cages

Calculating the Amount of Concentrated Paramecia Needed

While in the autonursery, baby zebrafish are fed 40mls of concentrated paramecia twice per day. Calculate how much concentrated paramecia you will need for both the morning and afternoon feedings based upon these numbers. The amount of paramecia that each cage 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 500ml 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 you daily food needs. The proper density for concentrated paramecia, as prepared for feeding fish, should be 100 paramecia/ml liquid.

Concentrated Paramecia Preparation

Paramecia need to be thoroughly filtered and rinsed before they can be concentrated and fed to the nursery. Pour each cage 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 15-20 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 23 micron strainer (see figure 2). The paramecia are too large to fit through the 23 micron material. Unlike the 105 micron strainer, the 23 micron strainer withholds the paramecia in the strainer and

discards the liquid. After the majority of the water is drained, using fish water, rinse the paramecia to remove any remaining ammonia.

After rinsing, pour the paramecia into a clean container. Fill the container with clean fish water until a concentration of 100-150 paramecia/1ml fish water is achieved and feed. Note: Typically before we feed the paramecia, we check the ammonia level with a test kit. If it shows any trace of ammonia, we repeat the 23um mesh straining process.

Modifications for Small Facilities

Small facilities may require a much smaller amount of food than the standard procedure yields. There are several modifications that can be made to the paramecia production process to meet the needs of a smaller facility.

Removing the Subculture Step:

One easy way to tailor the procedure to meet a smaller facilities needs, is to remove the subculture step. Instead of using culture plates to make subcultures and then using those subcultures to inoculate a large number of cages, each culture plate can be used to directly inoculate up to 4 cages. Remember to make enough Petri plates each week to make new cultures the next week, have at least two back up plates and make food cages. For example, if you are making 7 plate sets, 1 will be used for new plates, 2 should be saved for back-up, leaving 4 for making cages. Four plates will make 16 cages of paramecia.

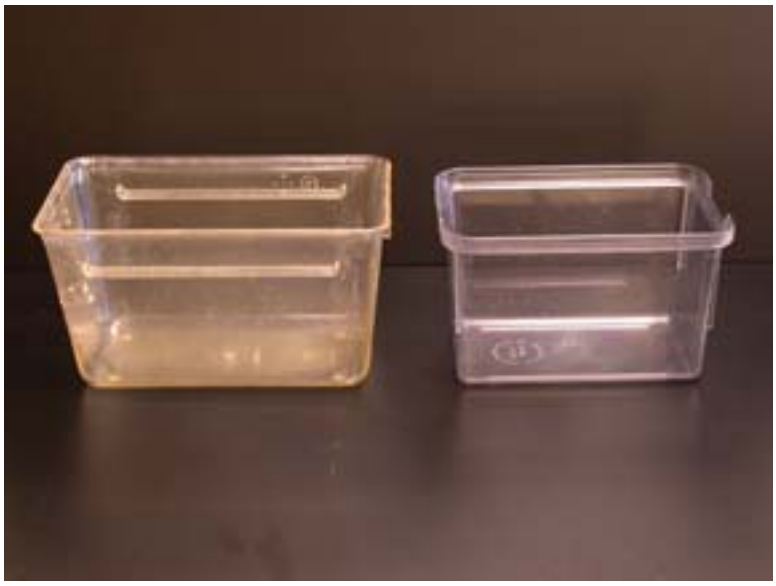
Petri Plates:

Facilities needing less than 16 cages of food per week can reduce the number of Petri plates made in each batch. This decreases the amount of space need for storing cultures, decreases the amount of supplies needed, and reduces the amount of labor required to maintain the plates. Making a fewer number of plates also decreases labor because most of the ingredients and supplies require autoclaving.

Cage Size:

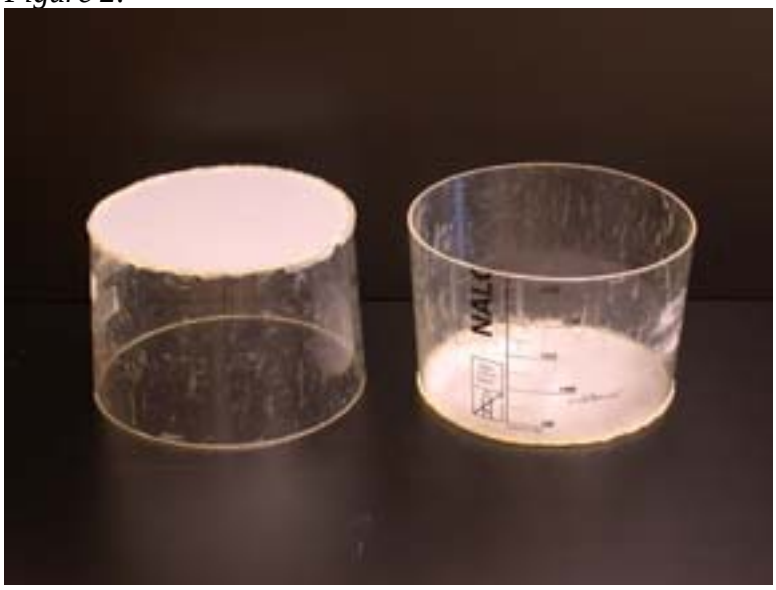
If 1 liter cages are too large for the daily needs of the facility, smaller cages can be made, as long as the recipe is adjusting accordingly.

Figure 1:



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. Both containers have been used successfully for paramecia at ZIRC and any similar containers should be adequate.

Figure 2:

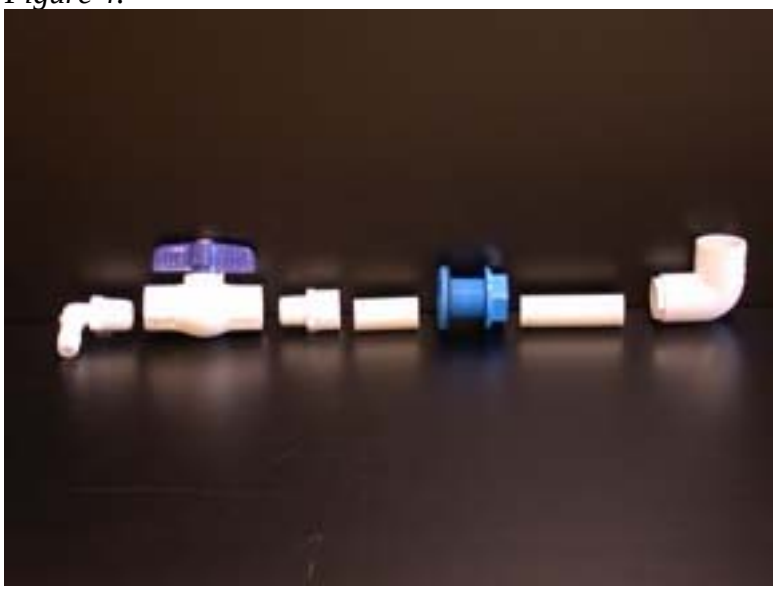


Strainers used in the paramecia making process are hand made by taking a large plastic beaker and cutting it into cylinders. Polyester filter cloth is then fixed to one side using hot glue.

Figure 3:

The paramecia collector is made from a carboy, with a spigot at the bottom, and various plumbing fixtures. A four gallon carboy is recommended for larger facilities, while a 2 gallon carboy will work well for those needing to make only small amounts of paramecia.

Figure 4:



The 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 what container you are using. 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.