University of Oregon
Zebrafish International Resource Center

TITLE: ZIRC Paramecia Procedure

SUBJECT AREA: Zebrafish Husbandry and Feeding

PROCEDURES:

List of Ingredients and Materials:

Cultures

- 200 to 2000 ml plastic containers with large surface area, or Petri dishes, at least 150x20mm (Figure 1)
- Dechlorinated filtered tap water, Nanopure, reverse osmosis, or deionized water
- Nutritional brewer's yeast (crushed and/or powdered)
- Autoclaved dry whole-wheat kernels

Other Items

- Measuring spoons, 0.05g
- Measuring spoons, 1 tbsp., 1/8 tbsp. and 1/4 tbsp.
- Warm room with medium light (24-26°C)
- Small kitchen tea strainer
- Sieves/strainers made from 105 and 10 μm polyester filter cloth (Figure 2)

Note: For the latest vendor information, please email us at zirc@zebrafish.org. Vendors are not listed in the procedure due to the high rate of unavailability.

List of terms:

Cultures (a.k.a. starter cultures):
Clean, but not sterile, paramecia grown in **Nanopure, reverse osmosis, or deionized water**. Cultures are inoculated using 1-3 week old cultures that are heavily concentrated with paramecia. The paramecium grown in these cultures are also fed to the larval fish.

Additional Information:

- Culture containers should hold 200 to 2000mls of water, have a large surface area, be easy to wash, and ideally able to withstand high temperatures so that they can be cleaned using a cage washer or high temperature dishwasher. A large water surface to air ratio is vital in growing dense paramecia cultures.

- Each culture will require approximately 1-4 weeks to reach optimal density/concentration.
- Constant temperature between 24-26°C is vital for steady growth. Temperatures outside of this range can slow the growth and can cause death of the colony.

Making Cultures

Introduction

When culturing paramecia, you are essentially creating an ecology in which microorganisms thrive. There are a host of other organisms besides paramecia (i.e. bdelloid rotifers, harmless to zebrafish larvae) 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) such as Coleps.

The cultures at ZIRC are routinely monitored for the presence of opportunistic organisms. A small percentage of bdelloid and vorticella rotifers are present in our colony. Rotifers are a known food source for zebrafish. While rotifers do not harm paramecia cultures, we occasionally perform serial dilutions on the ZIRC cultures in order to reduce rotifers levels. In your own facility, a serial dilution can be performed on established cultures at any time and will ensure the cleanliness of your colony if contamination occurs.

If you have questions regarding anything you see in your cultures or observe in your colony, please feel free to contact us at zirc@zebrafish.org.

Preparation

1. Autoclave dry, whole-wheat berries and store in sterile container.

2. Start with a clean/sterilized work surface. This will reduce the risk of contamination.

3. Bring 1 tbs of the autoclaved whole-wheat berries to a rolling boil for 10 minutes using Nanopure, reverse osmosis or dechlorinated water. After boiling, remove the wheat berries from the hot plate, pour off the excess liquid and wait a few minutes to allow the wheat to completely cool.

4. Prepare sterile Petri-dishes (containers) at least 150x20mm in size and label them with the date on the side and/or the top (Fig. 1).
   - Fill each dish with 125 ml Nanopure water or similar water source
   - Add approximately 0.01 grams of powdered brewer's yeast to each dish
   - Add 5 wheat berries to each Petri dish

Inoculation

1. Approximately 5 Petri dishes of concentrated paramecia culture (800 ml) will be needed to inoculate 20 new Petri dishes. Each starter culture should have a density of at least 100 cells/ml.

2. Sieve each starter culture through a kitchen (or tea) strainer to remove the old wheat berries. Collect the strained culture solution in a large beaker or bottle (1 L).
    - Use a squirt bottle filled with Nanopure water or other waster source to rinse and remove any paramecia attached to berries. Place the old wheat berries in the garbage after rinsing.

3. Divide the sieved starter culture equally between the newly prepared Petri dishes, adding at least 40 ml of inoculate per Petri dish. Gently mix the inoculating culture repeatedly during this process since paramecia tend to concentrate on the surface.

4. Cover each petri dish and label it with the date of the inoculation.

5. Maintain paramecia cultures between 24 and 26 ºC. Avoid temperature fluctuations, especially high heat; cultures grow best in constant conditions.

6. To reach optimal density, allow the cultures to reproduce and grow for approximately 1-4 weeks.

Notes:

- The inoculated paramecia cultures have a relatively long "shelf-life" that is ideal for adapting to changes in nursery feeding requirements.
- Dishes can be used for feeding as early as 1 week after inoculation and up to 4 weeks. After 4 weeks, the paramecia populations reach a stationary phase and tend to decline in density. Optimal feeding density is between 2 and 4 weeks.

Harvesting Paramecia from Cultures for Larval Feeding

Concentrated Paramecia Preparation

Filter and rinse paramecia thoroughly before concentrating and feeding to the larval fish.

Filter Step #1:

1. Pour each paramecia culture through a 105 μm sieve (See Figure 2) to remove the large particle debris (wheat berries and yeast).
2. Collect the sieve culture solution (paramecia) in a 5L container/pitcher.
3. Discard the debris collected in the sieve, rinse the sieve thoroughly with Nanopure water or similar waster source and store the sieve.

Filter Step #2:

4. Using a 10 μm mesh sieve (Fig. 2), restrain the paramecia from step 1 to remove the ammonia wastewater and retain/concentrate the paramecia. The paramecia

are too large to fit through a 10 μm filter and unlike the 105 μm strainer, the 10 μm sieve retains the paramecia and the flow-through wastewater can be discarded.

5. This process can be very slow as the paramecia and debris can clog the filter mesh. As needed, add Nanopure or similar water source to the sieve/paramecia to aid the rinse process.
6. When the majority of the wastewater has been removed, use a squirt bottle with Nanopure or similar water source to rinse the paramecia out of the sieve and into another container/pitcher.
7. Re-suspend the concentrated paramecia in fresh system water. Dilute to feeding concentration (100-150 cells/ml).

Notes:

- Feeding concentration: After rinsing, pour the paramecia into a clean container. Fill the container with clean fish water until a concentration of 100 to 150 paramecia/ml fish water is achieved.
- Before feeding the paramecia, check the ammonia level with a test kit. If it shows high traces of ammonia, repeat the 10 µm mesh straining process. To prevent the mesh sieve from clogging with debris, rinse using a high-pressure spray.

Figures:

Culture Containers/Petri Dishes

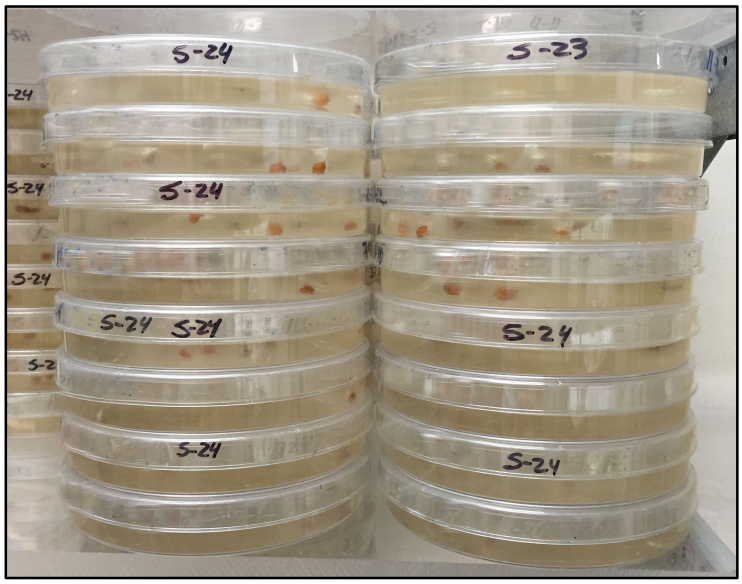

Figure 1. Example of the ZIRC Petri-dish cultures (150 X 25mm dishes). Any container with a large surface area can be used growing paramecia.

Micron Mesh Sieves/Strainers

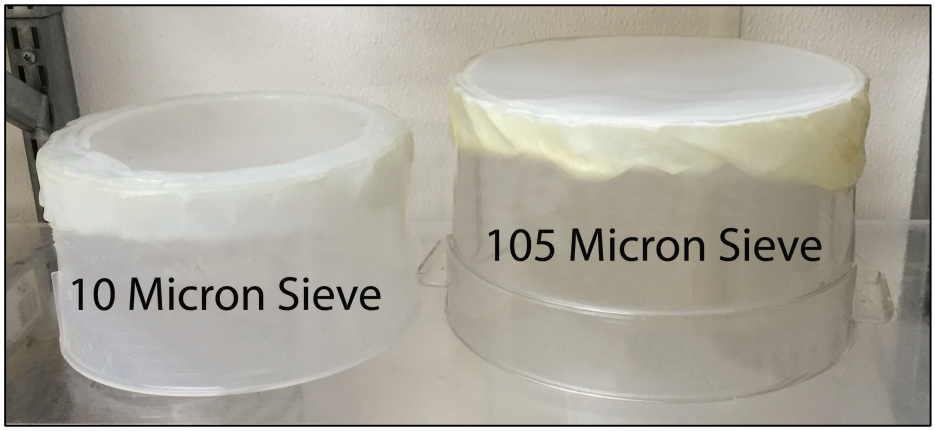

Figure 2. Mesh sieves used in the cleaning and concentrating of paramecia. The sieves are hand made by taking a large plastic beaker and cutting it into cylinders. Polyester filter or bolting cloth is then fixed to one side using hot glue. 10 μm and 105 μm mesh sizes.