University of Oregon
Zebrafish International Resource Center

TITLE: ZIRC Paramecia Procedure

SUBJECT AREA: Zebrafish Husbandry and Feeding

PROCEDURES:

List of Ingredients and Materials:

Ingredients

- Dechlorinated filtered tap water, Nanopure, reverse osmosis, or deionized water
- Nutritional brewer's yeast (crushed tablets and/or powdered)
- Autoclaved dry whole-wheat kernels/berries

Materials

- 200 to 2000 ml plastic containers with large surface area, or Petri dishes, at least 150x20mm (150x25mm ideal) (Figure 1)
- 100 ml beaker
- Measuring spoon, 0.05g
- Measuring spoon, 1 tbsp
- Small kitchen tea strainer
- Sieves/strainers – 55µm and 10 µm polyester filter (Figure 2)
    - Note: Alternative micron mesh sieves are available on-line, however the recommended a size range of 37-60 microns for large debris removal.

Environment

- Room temperature space with medium light (20-23°C)
    - Note: Cool temperatures are better than too warm.

Note: Current vendor information is provided below. If an item is no longer available, please email us at zirc@zebrafish.org.

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 larval zebrafish.

Additional Information:

- Culture containers should have a large surface area and be easy to wash. Ideally the container(s) should be able to withstand high temperature so it

can be cleaned using a cage washer or high temperature dishwasher. A large water surface-to-air ratio is crucial for growing dense paramecia cultures.
- Each culture will require approximately 1-4 weeks to reach optimal density/concentration.
- Constant temperature between 20-23°C is vital for steady growth. Temperatures outside of this range can slow growth or 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 rotifer 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 1-2 tbsp 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 allow the wheat to completely cool. Helpful note: Spreading the wheat into a single layer, allowing it to cool and dry is ideal. The use of a flat sieve is recommended.

4. Prepare Petri-dishes (containers) at least 150x20mm in size and label them with the date on the side and/or on top of the stack (Fig. 1).
    a. Fill each dish with 150 ml Nanopure water or similar water source
    b. Add approximately 0.01 grams of powdered brewer's yeast to each dish
    c. Add 15-20 wheat berries per dish

Inoculation

1. A single concentrated Petri dish/culture of paramecia (approximately 150 ml) can be split to make 3 new culture dishes. Each starter culture should have a density of at least 100 cells/ml. For larger batches:
    - 4 Petri dishes of concentrated paramecia = 20 new cultures
    - 8 Petri dishes = 40 new cultures

2. Sieve each starter culture through a kitchen tea strainer to remove the old wheat berries. Collect the strained culture solution in a large beaker (1000 ml).
    - Dispose of old wheat berries into the garbage or garbage disposal. Note: The old wheat berries are smelly, so a garbage disposal is ideal.

3. Divide the sieved culture solution equally between the newly prepared Petri dishes, adding approximately 30 ml of inoculate per Petri dish. Gently mix the sieved culture repeatedly during this process since paramecia tend to concentrate on the surface.

4. Cover each petri dish and label it or label an entire stack with the date of the inoculation.

5. Maintain paramecia cultures between 20 and 23ºC (68ºF -73ºF). Avoid extreme 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 2-4 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 2 weeks after inoculation and for up to 2 months. After 6 weeks however, the paramecia populations reach a stationary phase and tend to decline in density. Optimal feeding density is between 3 and 6 weeks.
    - If necessary, additional wheat berries and yeast can be added to cultures older than 4 weeks to sustain them for longer periods.

Harvesting Paramecia from Cultures for Larval Feeding

Calculations for Harvesting

- At ZIRC, most petri-dishes are harvested 3-6 weeks after inoculation. At this stage, a single petri-dish of paramecia can feed 100 larval fish (i.e. 500 larval fish would require the harvesting 5-6 petri-dishes).

Additional Housing and Feeding Calculations

1. At ZIRC, larval fish are transferred to housing tanks (up to 50 fish/tank) at 5-8 days post fertilization (dpf). The tanks are filled with 150 ml fish system water and receive no flowing water for the first 5 days. See the Larval Rearing SOP for additional details.
2. Paramecia feedings start the same day the fish are transferred, and the larval fish receive paramecia twice per day for 5 days ending at 8-13 dpf.
3. During these 5 days, each tank receives 10ml of concentrated paramecia (500-1500 paramecia/ml) in the morning and 10ml in the afternoon.
    a. Example: 100 larval fish are split into 2 tanks with 50 fish per tank. In the morning, prepare enough paramecia for both the morning and afternoon feedings.
        i. 2 tanks X 20mls = 40mls concentrated paramecia total for the day (500-1500 paramecia/ml)

Paramecia Harvesting

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

Filter Step #1:

4. Please a 55 µm sieve (or a sieve of similar size) (See Figure 2) on top of a beaker or bucket. Pre-wet the sieve before use for better drainage.
5. Pour each paramecia culture through the 55 µm sieve to remove the large particle debris (wheat berries and yeast) and capture the paramecia in the beaker or bucket. Approximately, 8 dishes can be collected at a time before the sieve clogs making it hard to drain.
6. Periodically discard the debris collected in the sieve.
7. When sieving is completed, the sieve should be sterilized using EtOH, bleach or dishwashing. The **EZ-Strainers** shown in figure 2 can withstand high temperature, 82°C (180°F).

Filter Step #2:

8. Using a 10 or 15 µm mesh sieve (Fig. 2), re-strain the paramecia from step 1 to remove the ammonia wastewater and retain/concentrate the paramecia. The paramecia are too large to fit through a 15 µm mesh and unlike the 55 µm strainer, the 10-15 µm sieve retains the paramecia and the flow-through wastewater can be discarded.
9. This process can be slow as the paramecia and debris can clog the filter mesh. Swirling the sieve and rubbing the bottom of the mesh with your hand or spraying the underside of the mesh with non-chlorinated water can dislodge the debris and speed up the filtering process.

10. When the majority of the wastewater has been removed, rinse/clean the paramecia by adding approximately 1 liter of Nanopure, dechlorinated tap water, or similar water source to the sieve and re-filter. Repeat this process 2-3 times to ensure that all the ammonia wastewater has been removed and the paramecia have been well rinsed.
11. After the final rinse, use a squirt bottle with dechlorinated tap water, Nanopure or similar water source to rinse the paramecia out of the sieve and into a clean container/beaker.
12. Re-suspend the concentrated paramecia in dechlorinated tap, RO or similar water to a volume of 20 ml per tank (10 ml per AM and PM feeding). Note, the final feeding concentration should also contain 500 – 1500 paramecia per ml , however the more paramecia per ml the better for optimal larval survival. Note: If needed, test for ammonia using a freshwater test kit. If it shows high traces of ammonia, repeat the 10 µm mesh straining process.

Figures:

Culture Containers/Petri Dishes

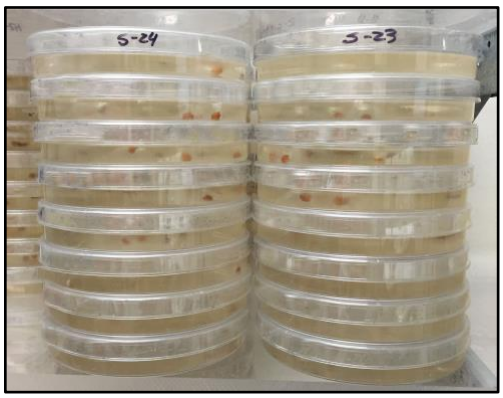

Figure 1. Example of the ZIRC Petri-dish cultures (150 X 25mm dishes). Any container with a large surface area can be used growing paramecia.

Micron Mesh Sieves/Strainers

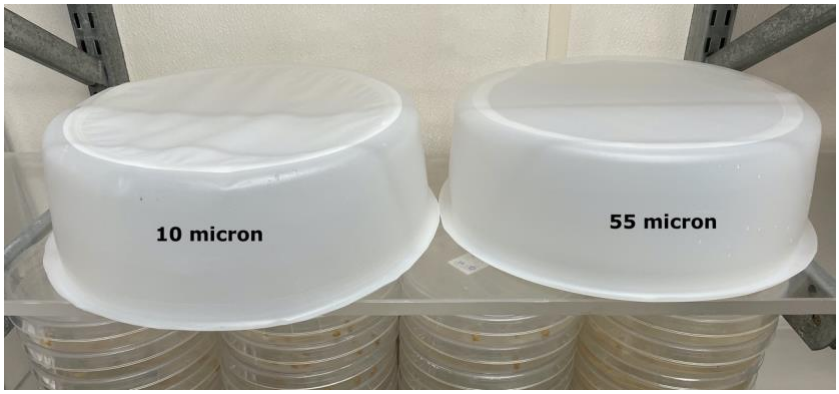

Figure 2. Mesh sieves used in the cleaning and concentrating of paramecia. A10 µm and 55 µm EZ-Strainer can be obtained from various on-line vendors. To speed the sieving process, a large sieve diameter is recommended. See vendor information below or contact, zirc@zebrafish.org for questions.

Vendor Information:

1. Petri dishes (150mm X 25mm):
   - Fisher Scientific, https://www.fishersci.com
     - Case of 60, Catalog number - 08-772-25
   - Millipore Sigma, https://www.sigmaaldrich.com
     - Pack of 5, Catalog number - CLS430599-5EA
     - Case of 60, Catalog number - CLS430599-60EA

2. Measuring spoon (0.05 gram):
   - Preiser Scientific, https://www.preiser.com
     - Catalog number - HAH-49200

3. Tea strainers
   - Amazon.com or other retail internet store
     - 3 to 3.75 inch Nylon Mesh Strainer

4. Sieve Options:
   - **55 Micron EZ-Strainer** for 5-gallon Containers/Buckets (ZIRC recommended)
     - Amazon, https://www.amazon.com
     - Duda Diesel, https://www.dudadiesel.com
       - Dishwasher safe at high temperature, 82℃
   - **10-Micron EZ-Strainer** for 5-gallon Containers/Buckets (ZIRC recommended)
     - Amazon, https://www.amazon.com
     - Duda Diesel, https://www.dudadiesel.com
       - Dishwasher safe at high temperature, 82℃
   - Multiple Sizes:
     - https://www.aquaculturenurseryfarms.com/sieves-plankton-collectors/sieve-stackable-pro-4/
       - These sieves often do not survive dishwashing

5. Plastic Pail (3.5 gallons) For Use with EZ-strainers
   - ULINE, https://www.uline.com/Product/Detail/S-9942W/Pails/Plastic-Pail-35-Gallon-White?keywords=S-9942W
     - Dishwasher safe at high temperature, 82℃

6. Brewer's Yeast
   - Amazing Nutrition Brewer's Yeast
     - https://www.gnc.com
     - https://www.amazon.com
   - Crush yeast tablets into powder using a mortar and pestle

7. Wheat Berries
   - Obtain from a local grocery or health food store, Amazon or other on-lines source
     - Via Amazon: Palouse Brand Hard Red Wheat Berries
   - Place in a glass bottle and autoclave prior to use