

Rotifer Culture, Harvesting, and Fish Feeding

Overview

The lab uses rotifers^{1,2} (*Brachionus plicatilis*) as a food for larval zebrafish. As the entire research program depends on efficient rearing of zebrafish through the larval period, it is absolutely critical that a constant supply of high quality rotifers is on hand at all times. The following instructions must be followed exactly, without deviation. Comments and suggestions are welcome but must not be implemented until they are considered and formally incorporated into the protocol used by everyone in the lab.

Rotifers are on a four-day cycle. They are raised in plastic 24-quart liquid dispensers³, referred to as “tanks”. The most recently inoculated tank is referred to as “Day 0”, the previous day’s tank as “Day 1”, etc. Tanks of rotifers contain “rotifer water” made by adding Instant Ocean salts to water purified by reverse osmosis filtration.⁴

We use marine rotifers because the cultures are more robust than those of freshwater rotifers, and the marine rotifers live in our standard zebrafish larval water.⁵ We use RO water as our starting point for making rotifer water because even dechlorinated Seattle tap water is toxic to the cultures (probably due to heavy metal contamination), though this is not true for all locations.

This protocol covers the daily care and maintenance of the rotifer cultures, harvesting and inoculating rotifer cultures, and the feeding of harvested rotifers to larval fish. Each of these tasks is detailed separately and a protocol for integrating all of the tasks is included at the end.

Daily feeding and monitoring of the rotifer tanks

- Rotifers are fed and checked at least four times daily. At each feeding, verify that the rotifers have eaten and that the cultures are healthy; if there is a problem, the culture will appear a turbid green; if rotifers have consumed their food from the last feeding, the culture will appear more translucent and brown. Also verify that air hoses are bubbling in each tank.
Overfeeding can lead to fungal growth and can kill the cultures. If there is any doubt whether rotifers are eating, check with senior lab personnel before adding more food. Air bubbling should be at a low or moderate level, and should not be too vigorous.
- At least once per day, examine a small aliquot from every rotifer tank under the microscope. To sample the rotifers, use a plastic transfer pipette and place 1 ml from each tank into separate wells of a multi-well culture plate. Check each well under a stereomicroscope for rotifer health and numbers. Healthy rotifers will be swimming rapidly and should have multiple attached eggs. Unconcentrated samples should contain 20–100 or more rotifers. If rotifers are lethargic, lack eggs, or are few in number, there is a problem; inform Dave immediately. Check also the tanks pH, using the portable pH meter or test strips. The pH should be 7.0–7.6; if outside this range, check with Dave.
- Assuming cultures are healthy, they are fed concentrated Instant Algae⁶ and supplemented with ChlorAm-X⁷ powder. The table below gives the amount of Instant Algae and ChlorAm-X to be added at each feeding. Instant Algae is

added with a graduated transfer pipette and ChlorAm-X powder is added using the blunt end of a small metal spatula (~300 mg/scoop). Beakers of harvested rotifers are fed the same as Day 0 tanks. After adding the Instant Algae and ChlorAm-X, stir the culture with the plastic stirrer, proceeding from the youngest tank to the oldest.

ChlorAm-X neutralizes ammonia, a harmful metabolic byproduct, and is absolutely essential for healthy cultures.

Rotifer feeding and supplementation

Instant Algae

	Tank			
	Day 0, H *	Day 1	Day 2	Day 3
early morning	10 drops	1 ml	2 ml	2 ml
late morning	10 drops	1 ml	2 ml	2 ml
mid-afternoon	10 drops	1 ml	2 ml	2 ml
late afternoon †	1 ml	1.5 ml	3 ml	3 ml

ChlorAm-X powder

	Tank			
	Day 0, H *	Day 1	Day 2	Day 3
early morning	1 spatula	1 spatula	2 spatulas	2 spatulas
late morning	1 spatula	1 spatula	2 spatulas	2 spatulas
mid-afternoon	1 spatula	1 spatula	2 spatulas	2 spatulas
late afternoon †	1.5 spatulas	1.5 spatulas	3 spatulas	3 spatulas

* H, Harvested rotifers in plastic beakers. Note: Harvested rotifers are fed rotimac and Instant Algae 1 hour before they are fed to the fish; see below.

† The last feeding of the day is increased to satiate the rotifers overnight.

Daily harvest and inoculation of a new tank

1. Gather together several clean 4 liter plastic beakers. Label one beaker with the inoculation date (today), the harvest date (4 days from today), and your initials; this is the “*inoculate*” beaker and will receive the highest quality rotifers for continuing the culture. Label another beaker to hold the rotifers that will be fed to the fish; this is the “*fish*” beaker and its contents will be split into two batches, one to feed the fish this evening, and one to feed the fish tomorrow morning.
2. Drain ~2 gallons from the oldest (Day 3) tank into two of the unlabeled beakers.
3. At the sink, place the 105 µm screen over another empty, unlabeled beaker and pour the first gallon of Day 3 rotifers through the screen, catching the flow-through in the beaker. Using a squirt bottle containing rotifer water, rinse the rotifers off of the 105 µm screen into the *inoculate* beaker. Then, place the 23 µm screen over another clean beaker. Pour the filtrate from the 105 µm screen through the 23 µm screen, catching the flow through in the beaker. Rinse the rotifers off of the 23 µm screen into the *fish* beaker. You will now place the 5 µm screen over another clean beaker and pour through it the 23 µm filtrate. Add 1 liter of the resulting 5 µm filtrate to the inoculate beaker and discard the remaining 5 µm filtrate.

Only the largest rotifers that are caught on the 105 μm screen are used for the inoculate because they are most likely to carry eggs and they are the least likely to have contaminating protists and other microorganisms. Providing them with 1 liter of the 5 μm filtrate helps prevent osmotic and other shocks; the extra filtration step is essential to prevent transmission of contaminants in the cultures.

4. Repeat the above procedure with the second gallon of Day 3 rotifers, rinsing the 105 μm rotifers into the *inoculate* beaker and the 23 μm rotifers into the *fish* beaker. Do not keep the 23 μm filtrate this time.
5. Inspect a small sample of rotifers from the inoculate beaker under the microscope to be sure they are healthy. Then fill the inoculate beaker to 4 liters with rotifer water and pour the contents into a clean rotifer tank; this is the new Day 0 tank and will be placed on the rack when the Day 3 tank is removed. *We fill the Day 0 tank only to 4 liters to maximize the encounter rate between rotifers and their food during the early phase of the culture.*
6. The rest of the harvest can be accomplished by simply pouring rotifers one gallon at a time through the 23 μm screen, with the filtrate allowed to go down the sink, and the rotifers rinsed off the screen into the *fish* beaker. Harvest all of the remaining rotifers in the Day 3 tank, including the bottom of the tank. Also harvest two gallons each from the Day 1 and Day 2 tanks. Inspect a small sample of rotifers from the *fish* beaker under the microscope to assess health and numbers.
7. You will now finish preparing the rotifers that will be fed to the fish. To do this, fill the fish beaker with rotifer water and pour half of the rotifers into a new clean 4 liter beaker. Top off both beakers so they are ~two-thirds with rotifer water. Label one "AM" and one "PM" and place both on rotifer rack. Add an air bubbler to the AM beaker. *The beaker is filled first to suspend the rotifers so they can be divided more accurately.*
8. Fill the previously untouched Day 1 tank, as well as the harvested Day 2 and Day 3 tanks with rotifer water. Place the newly inoculated Day 0 tank on the rack.
9. Immediately feed all of the rotifer tanks and beakers.

Feeding rotifers to the larval fish

1. One hour before feeding the larval fish, feed the appropriate ("AM" or "PM") rotifers with 5 ml rotimac⁸ and 15 drops of Instant Algae. *Feeding the rotifers before they are given to fish is absolutely essential. The rotimac contains essential fatty acids, vitamins, etc. and is a major source of nutrition for the fish larvae. Think of the rotifers themselves as a package for delivering these nutrients; the rotifers must have enough time to feed but not so much time that they process the food.*
2. At feeding time, pour the appropriate rotifers through the 23 μm screen and rinse the rotifers off the screen into a small beaker using rotifer water. Fill with ~150 ml rotifer water.

A quick re-screening keeps the larval fish water cleaner and helps to maintain a high quality rearing environment, thereby reducing fish mortality.

3. Determine how many fish tanks and fish beakers need rotifers and aliquot rotifers to them accordingly. Be sure to keep the rotifers suspended by gently swirling their container. Generally, beakers should receive from 2–3 drops of rotifers (for newly hatched larvae) to as much as 0.5 ml (for older larvae). Tanks of fish should generally receive 1–5 ml of rotifers but all rotifer amounts should be scaled according to harvest volume and fish number. Check with Dave or Cindy for details.
4. At least once per day, fish in beakers or in tanks without flowing water are given diluted ChlorAm-X to reduce ammonia levels. A stock solution⁹ is kept in the fish room for this purpose. Use the repeating pipettor to administer 100 μ l ChlorAm-X to each beaker and 300 μ l ChlorAm-X to each tank.

Daily protocol

Following is a suggested protocol for accomplishing all the required rotifer-related tasks. As written, it is easily integrated with daily Fish Maintenance. While the exact timing can be changed according to one's schedule, please note:

- **Rotifers must be fed early enough so that larval fish can be fed by 10 am.**
So...one should start no later than 9:00 am.
 - **Rotifers should receive their last feeding no earlier than 4 pm, and fish should receive their last rotifers no earlier than 5:00 pm.**
1. First thing in the morning (~8 am) check the health of all rotifers. Make rotimac⁷ solution. Feed the rotifer tanks (Instant Algae + ChlorAm-X). Feed the beaker of AM rotifers (5 ml rotimac + Instant Algae).
 2. After one hour, screen the AM rotifers and feed them to the fish; administer ChlorAm-X to the fish, as described above.
 3. During mid to late morning (~11 am), feed the rotifers again.
 4. In the early afternoon (~2 pm), harvest the rotifers as described above. Feed them.
 5. In the late afternoon (~5 pm), feed all the rotifer tanks and the AM rotifers (Instant Algae + ChlorAm-X). Feed the PM rotifers (5 ml rotimac + Instant Algae).
 6. After one hour, screen the PM rotifers and feed them to the fish.

Notes:

¹ For more information see: Hoff F. H. and Snell T. W. 2004. *The Plankton Culture Manual, 6th Edition*. Florida Aqua Farms, Inc.

² High quality starter cultures of marine rotifers can be obtained from Reed Mariculture (<http://rotifer.com>, <http://www.reed-mariculture.com>). On receiving the rotifers use the following procedure to avoid osmotic and other shocks (from the Reed Mariculture website):

- i. Open the box and store the bags of rotifers in a refrigerator (4-8 C) until you are ready to put them in the rotifer tank.
- ii. Measure the pH in the bag of rotifers (it will probably be about 7) and adjust the pH in your culturing tank to be the same. Otherwise the rotifers can be pH "shocked" and many of them can die. The pH will naturally return to its normal values within several hours and the rotifers will adjust without any problems. For information about adjusting pH click [here](#).
- iii. The rotifers will arrive at a salinity of roughly 20 ppt (1.015). Your water does NOT need to be adjusted to this salinity - they will go into water from 20 to 30 ppt (1.023) without any problems.
- iv. Add the recommend amount of ClorAm-X or Ultimate to the tank to buffer the water. Dosage rates can be found at www.cloram-x.com.
- v. Take the bag of rotifers from the refrigerator and put it in the rotifer tank for 10-15 minutes to allow it to slowly warm up. Then cut the bag open and release the rotifers into the tank.
- vi. Add enough Nannochloropsis or "Rotifer Diet" to the water to establish a green background.
- vii. Check the rotifer tank in 2 hours to see if more algae is needed. The rotifers will be hungry after their trip and will consume more than usual amount for the first few hours.

³ <http://www.servu-online.com> #PRLQ-2000

⁴ Rotifer water is made by dissolving 280 g Instant Ocean salt per five gallons of RO water.

⁵ Zebrafish larval water is made by dissolving 168 g Instant Ocean salt per 10 gallons of RO water.

⁶ Instant Algae is non-living, preserved *Nannochloropsis* available from Reed Mariculture. It is stored frozen at -20 °C until needed, and then kept in aliquots at 4 °C.

⁷ ChlorAm-X is purchased in bulk from Reed Mariculture, though it can also be obtained from Aquatic Ecosystems if necessary. We normally keep one 50 lb container on hand.

⁸ Rotimac is obtained from AquaFauna BioMarine (<http://www.aquafauna.com>). For rotimac solution, add 0.5 g rotimac powder to 10 ml rotifer water and shake well. Store in the refrigerator and use for one day only.

⁹ Larval fish ChlorAm-X solution is made as: 5 g ChlorAm-X in 100 ml RO water.