

Rotifer High Density 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 available 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 raised in high density culture tanks (aka “factories”) containing “rotifer water” that is made by adding Instant Ocean salts to water purified by reverse osmosis filtration.³ 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.⁴ *We use RO water as our starting point for making rotifer water and larval water because even dechlorinated Seattle tap water is toxic to the cultures.*

Water quality in the rotifer factories is maintained by: partial, daily water exchanges; aeration; a protein skimmer to remove excess organic material; and supplemental dosing with a solution of ChlorAm-X to ensure ammonia is removed and sodium bicarbonate (baking soda) to control pH. Rotifers are fed a preserved algae paste while they are in the factories. Both ChlorAm-X/bicarb and algae paste are delivered automatically by peristaltic pumps throughout the day and night; the two together determine the number of rotifers produced each day.

Healthy, well-fed rotifers are reasonably nutritious for larval fish. However, we increase the nutritional value of harvested rotifers by feeding them Rotimac—which is high in vitamins, omega fatty acids, and other nutrients—about one half hour before providing them to the fish.

This protocol covers care and maintenance of the rotifer cultures and systems, harvesting rotifers, and feeding of the harvested rotifers to 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 factories

Factories must be checked daily to verify culture health, algae and ChlorAm-X/bicarb dosing, mechanical components, and water quality. If there is any doubt about culture health or system functions, immediately consult with Dave (cell: 206 734-7331) or other senior lab personnel. At each check:

1. Verify that the rotifers have eaten and that the cultures are healthy. Healthy rotifers will be fast moving with attached eggs. Examine the color and turbidity of the cultures: healthy cultures are a relatively clear green/brown color. Turbid green implies overfeeding and possibly a crash in rotifer numbers. Clear brown to red implies inadequate feeding and starving rotifers, and also a potential crash. Take a pipette-full of rotifers and assess numbers, motility, and egg production; record your observations.

If there are few rotifers in the cultures, check with senior lab personnel about how much

to harvest. Also check to see if the protein skimmers are producing foam at a normal rate. Do not increase the algae drip to feed more to the rotifers, as this can easily cause the cultures to crash if the filtration is inadequate.

2. Check the aeration. All three air diffusers in the rotifer tanks should be bubbling gently and the aeration ring around the center filter should be bubbling as well, resulting in a “gentle boil” for the culture overall. The two air valves for the biological filter tank should be open fully and the diffusers bubbling vigorously. *Aeration in the rotifer tank should be sufficient to keep the culture suspended but not so vigorous as to damage the rotifers. Aeration around the center filter is essential to keep it free of debris; inadequate aeration can result in clogging, which will overflow the tank! We have one extra air pump should either of the two current pumps fail.*
3. Verify that the peristaltic pumps and timers are working for algae and ChlorAm-X/bicarb dosing. Turn off and on the powerstrips that control the timers to reinitiate a dosing cycle. Verify that algae and ChlorAm-X/bicarb drip into the rotifer tank and are not caught on air hoses, pipes, etc. *Since the cultures are run at their carrying capacity based on algae and ChlorAm-X/bicarb dosing, any problems with the pumps, timers, or feed lines can quickly cause the cultures to crash! Note that we have one extra peristaltic pump should any of the four current pumps fail.*
4. Verify that the algae and ChlorAm-X/bicarb reservoirs are sufficiently filled and record the volumes remaining on the data sheet⁵. If algae or ChlorAm-X/bicarb must be added, first check that mold or other debris have not accumulated in the bottles and swap-out the bottles if necessary. Make sure the algae cooler is functioning properly. *If the ChlorAm-X/bicarb solution has run-out or if the pumps have failed, check the pH of the rotifer tank and adjust with sodium bicarbonate powder if necessary (typically ~half tablespoon sodium bicarbonate per 0.2 pH units). Normal pH should be 7.5–8.5.*
5. Check the water levels in the tanks. Water lines in the rotifer tank and biological filter tank should be at the same level. *If the water level in the rotifer tank is higher than it should be, if the water level in the biological filter tank is lower than it should be, or if both are occurring, this means that water is not being returned properly to the biological filter tank. The most likely explanations are: (i) clogging of the center mesh filter in the rotifer tank: close valve #3 to isolate the rotifer tank, remove the current filter and replace with the spare; (ii) too rapid flow from the protein skimmer into the rotifer tank: close valve #1 to further restrict the flow; (iii) clogging of protein skimmer apparatus, which reduces overall flow to the skimmer and will push it to the main tank; (iv) some combination of the above.*
6. Check the protein skimmers and collection buckets. The water level should be just below the bottom of the clear portion of the skimmer and there should be plenty of foam being produced. The buckets should not be overflowing. *Be sure the water level is stable and not too high. Open and close valve #2 to change the water level in the skimmer; the valve is very sensitive: small changes (~1 mm) dramatically affect the pressure and resulting water level: too much can cause the system to overflow through the skimmer into the spill palettes. Important: It is critical that skimmers are producing foam. If they are not, the cultures may crash owing to protein and bacterial accumulation. Under these circumstances,*

check with Dave or other senior lab members about cleaning the system and reducing the algae drip, which will help to avoid overloading the culture with biological material under these conditions.

7. In the event of a major spill or other mechanical problem that cannot be remedied as described above: assuming there are rotifers to save, isolate the rotifer tank by closing valve #3, unplug the yellow water circulation pump and leave the culture with aeration and oxygen only. Contact Dave immediately.

Harvesting rotifers

Rotifers are harvested once per day, typically beginning about 7–8 am. The harvesting itself takes about 20 minutes (with experience); note that filters are cleaned and mechanical systems checked and this requires about 15 minutes additional.

Important: *The spill palettes on which the factories sit can become wet during various steps in this procedure. The deck and especially the sides are slippery when wet and present a potential hazard. Be sure to watch your footing when you are working on the pallets and keep extraneous items off the pallets at all times.*

1. Gather the two 23 μm rotifer screen filters (one for each rotifer factory) and pre-wet them with tap water. Place the hose ends into the 23 μm rotifer screen filters. Check that the hose valves are off.
2. Close valve #1 (skimmer return to rotifer tank) and close valve #5 (biological filter isolation).
3. Remove the center filter from its hole in the rotifer tank. If cleaning the filters (see below), place the center filter and the three filter pads in a clean bucket. If not cleaning the filter, just leave the center filter in the tank.
Removing the center filter is necessary to allow the rotifers to drain from their tank.
4. Open valve #6 (main factory drain). Open the hose valves and collect the rotifers on the 23 μm rotifer screen filters. If the filters start to overflow, close the hose valves, tap the bottom of the screen, and use rotifer water to wash the collected rotifers into a beaker; continue the harvest. Typically we harvest 20%–40% of the rotifer tank at each harvest; ask Dave or other senior personnel what the appropriate volume will be for any given day if you are uncertain (e.g., if rotifer numbers are low). When the appropriate amount has been harvested close the hose valves and close valve #6.
5. Once the rotifers have been collected on the screens, wash them into a 5 gallon drink dispenser.
6. Wipe out the inside of the each rotifer factory with a clean piece of rotifer filter or clean sponge; be sure to clean off the sides both above and below the water line, as well as the bottom and the airlines and airstones. This helps reduce bacterial build-up in the system. If needed, do the same for the smaller tank (without rotifers).

7. Adjust valve #2 so the water level in the protein skimmers is below the clear tube at the top of the skimmer. Then remove the clear tube and its upper white top and wash out any accumulated foam under hot tap water; wash out the long collecting pipe if necessary. Replace the parts and readjust valve #2 so the water level is once again just below the clear tube. This is important to maintain skimmer efficiency and culture health.
8. We aim to replace 50% of the water in the main culture tanks, regardless of what proportion of rotifers has been harvested. Therefore we replace the center filter and open valve #6 to drain the water until the tank is only half full (while keeping the remaining rotifers inside). Then, close valve #6 and replace the filter pads. Check that air lines are in place (one air diffuser in each third of the tank; air ring around center filter).
9. Add fresh rotifer water to the fill line of each rotifer tank and add rotifer water to fill the drink dispenser of harvested rotifers about two-thirds full.
10. Re-open valve #1 and re-open valve #5. Valve #1 should deliver as much flow to the main rotifer tank as possible, without overflowing the tank. Valve #5 should be fully open. Recoil the hoses and put them out of the way..
11. To the drink dispenser of harvested rotifers, add 20 ml of rotimac powder⁶ which has been thoroughly dissolved into rotifer water, plus 3 ml of ChlorAm-X/bicarb solution. Add the air bubblers.
12. Clean the filters by placing the center filter in the sink and use a strong jet of moderately hot tap water from the hose to flush out debris. Do the same for the filter pads. Rinse pads with RO water and return them to the tanks; leave the center filters to dry for the next day. Make sure these items are cleaned before they have a chance to dry.
13. Refill the rotifer water tank by adding the appropriate amount of RO water and salt; turn on the pump to mix.

Feeding rotifers to the larval fish

1. Thirty to sixty minutes after feeding Rotimac to the harvested rotifers, they can be fed to the larval fish. Pour about one-third of the harvested rotifers through a 23 μm rotifer screen filter. Wash them into a beaker and fill with ~200 ml rotifer water. Add 2 ml of 10% ChlorAm-X.
Waiting for the rotifers to eat Rotimac is absolutely essential, as the Rotimac contains essential nutrients for the fish. 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. A quick re-screening of the rotifers keeps the larval fish water cleaner and helps to maintain a high quality rearing environment, thereby reducing fish mortality. We add ChlorAm-X to the rotifers to reduce ammonia levels in the larval fish tanks.
2. 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, tanks on static water should receive from 4–5 drops of rotifers (for newly hatched larvae) to as much as 1 ml (for older larvae). Try to disperse the rotifers across the tank. Tanks of fish should generally receive 1–8 ml of rotifers but all rotifer amounts should be scaled according to harvest volume and fish number. Fish that are on flowing water and receiving only rotifers should receive relatively large amounts (e.g., two full pipettes). While such small fish will only be able to eat a small number of the rotifers actually delivered, we try to maximize their encounter rates given the rotifers will die quickly in the system water and will be flushed out owing to the water flow. The same is not true for fish in static larval water, in which rotifers live longer and are not flushed out of the tank (necessitating care so as not to feed so much as to foul the water). Check with Dave for details. If there are left-over rotifers after the feeding and they are still healthy, they should be returned to the rotifer factories (typically split evenly between the two factories).

3. For midday feedings, rescreen half of the remaining rotifers, as above, and feed to the fish. For the afternoon feeding, verify under the microscope that some rotimac remains in the water and the rotifers are well-fed; assuming this is the case, rescreen the remaining rotifers and feed them to the fish. If the rotifers are hungry, give them a supplemental amount of rotimac (scaled down from normal), let them eat for 30–60 min and then rescreen and feed to the fish. For both midday and afternoon feedings remember to add ChlorAm-X/bicarb to the collected rotifers before they are taken to the fish room. Return unused rotifers to the factories.

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 (see **Fish Maintenance SOP**). 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 9:30 am.**
So...one should start no later than 8:45 am.
 - **Rotifers should receive their last check no earlier than 4 pm, and fish should receive their last rotifers no earlier than 4 pm.**
1. First thing in the morning (~8 am) check the health of all rotifers and perform the harvest, cleaning, etc. Verify the algae and ChlorAm-X/bicarb reservoir levels and supplement or replace as needed. After 30–60 min, screen the morning rotifers and feed them to the fish.
 2. At midday (~12 pm), prepare and feed the harvested rotifers for the supplemental feeding and give to the fish.
 3. In the afternoon (~4 pm), prepare and feed the harvested rotifers as described above and give to the fish.

4. At the end of the day, perform the complete afternoon rotifer check and make sure that materials are clean and solutions replenished for the next day.

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

⁴ Zebrafish larval water is made by dissolving 168 g Instant Ocean salt per 10 gallons of RO water.

⁵ If algae reservoirs are low, refill with prepared algae paste. If ChlorAm-X/bicarb reservoirs are low, refill with 10% ChlorAm-X/bicarb solution [10% w/v ChlorAm-X, 5% w/v sodium bicarbonate (baking soda) in RO water: fill 5 gallon drink dispenser to ~4 gallons with RO water, add 2 kg ChlorAm-X and 1 kg sodium bicarbonate; fill to 5 gallons and stir into solution].

⁶ Rotimac is obtained from AquaFauna BioMarine (<http://www.aquafauna.com>). Store in the refrigerator or freezer.

