

Rotifer Production System Operation and Assembly Instructions (Part Nos. AR150K-AR1000K)



Part No. _____ Serial Number _____ Date Purchased _____



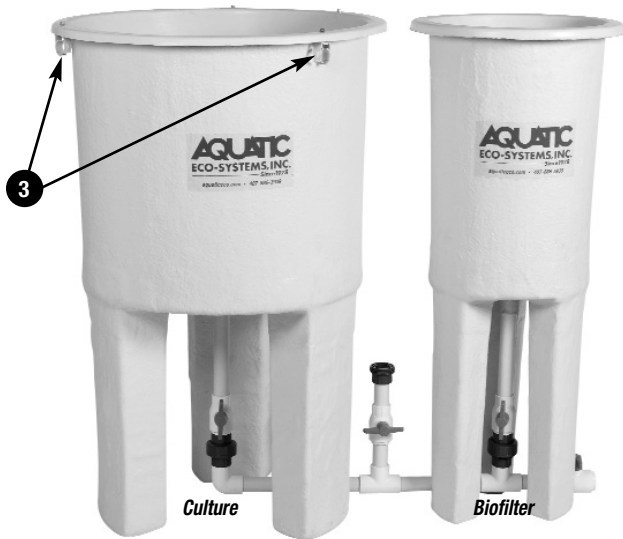
AQUATIC ECO-SYSTEMS™

Contents of Kit

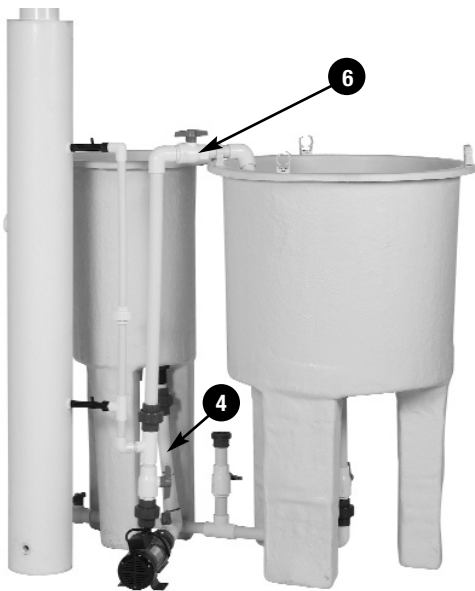
- Fiberglass Tanks (2)
- Protein Skimmer
- Oxygen Flowmeter
- Water Pump
- Diffusers
- Biofilter Media
- Peristaltic Pump
- Timer
- ClorAm-X®
- Tubing, Piping and Valves

Assembling the Rotifer Production System

1. Place culture tank on left and biofilter tank on right.
2. Attach PVC drain line to the bottom of both tanks. The line's center valve should be facing upward between the two tanks.



3. Unscrew pipe clips from under tank rims and replace in an upright position.



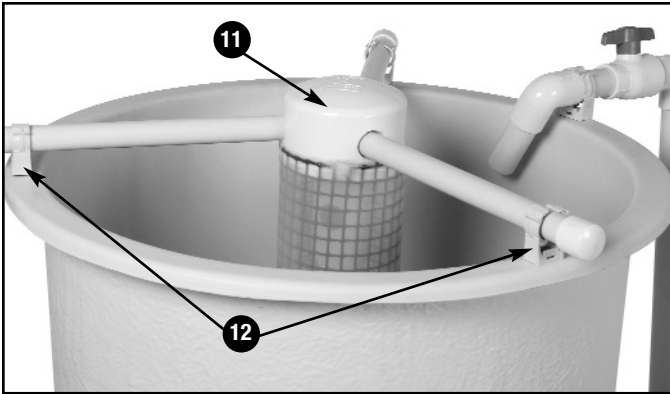
4. Fasten one end of the L-shaped pump supply line to the rear of the biofilter tank and the other end to the pump inlet.
5. Connect outlet of pump to protein skimmer using unions.
6. Attach pump return line to top union on skimmer line, with open end inside culture tank.



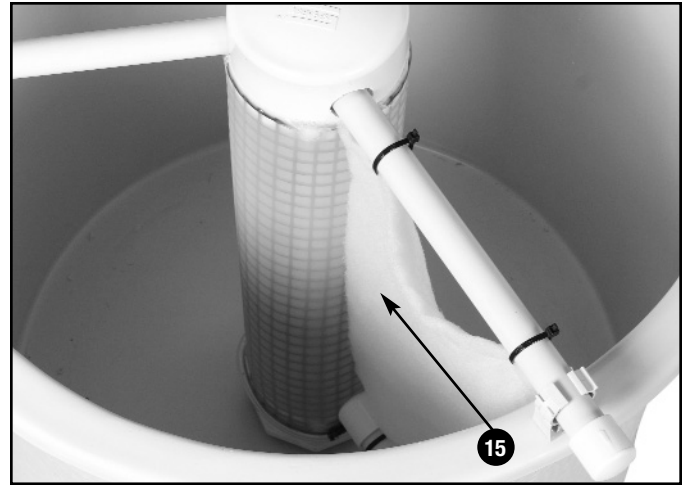
7. Secure skimmer return line to protein skimmer outlet, with open end inside biofilter tank.
8. Slide waste line, collection cup first, into top of protein skimmer.
9. Screw drain valve to bottom of skimmer.



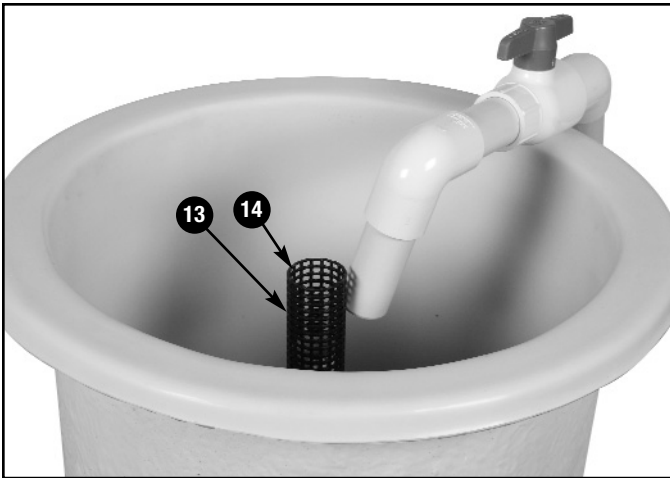
10. Attach harvesting port to center valve of drain line.



11. Insert large standpipe with bubble ring attached into hole in center of culture tank.
12. Fit the three filter pad hangers into the holes in the large standpipe and secure to the culture tank using the upright pipe clips. From above, these should look like spokes.



15. Cut filter floss to size (width should be approximate length of hanger). Using zip ties, attach one end of the cut floss to a weight and the other end to a hanger. Repeat for all three hangers.



13. Insert biofilter standpipe into hole in center of biofilter tank.
14. Screw pump intake screen into the bulkhead inside the biofilter tank.



16. Attach diffusers to diffuser manifold via provided air line and place three at the bottom of each tank. One diffuser should be in each sector formed by the hangers.
17. Add biomedica to biofilter tank (not pictured).

Your high-density rotifer system is now ready to use.

High-Density Rotifer Production System

Pentair Aquatic Eco-Systems, Inc., has developed a high-density, continuous, rotifer culture system that can maintain as many as 10,000 rotifers per ml. The system requires less space and labor than traditional batch culture systems. A variety of sizes are available, including a 150-liter system for labs and smaller applications and 450- and 1,000-liter systems for larger applications.

A Brief History of Rotifer Culture

In the past, rotifers were grown on live algae, either in ponds or in tanks, for use as a larval diet. Farms with space and water available to use traditional pond rearing methods should continue to do so. However, farms using live algae and tanks to grow rotifers should consider an alternative. Traditional batch culture requires a farm to have both live algae cultures and rotifer rearing tanks to provide feed for larvae. The table below shows a comparison of the typical requirements for both methods in a hatchery that is raising 500,000 marine fish larvae and needs 100 million rotifers per day.

*Information on batch culture was compiled from past experience at Aquatic Eco-Systems, Inc.

***Nannochloropsis Instant Algae Paste equal to 3,600 liters of culture at 19 million cells per ml.*

The batch method requires a much higher investment in equipment and space for both algae and rotifer production. The high-density method requires only rotifer tanks and the space for their filtration systems. The table does not account for the loss of water that occurs using live algae to grow rotifers. Generally speaking, the batch culture method uses a 75–100 percent exchange per day, with the addition of live algae and harvest. The high-density method uses algae paste and only requires between 20–50 percent exchange of water per day. This water exchange occurs when the tank is being harvested. Another factor is labor. Batch culture methods require at least two full-time live feed technicians, while the high-density method only requires an hour of a hatchery technician's time.

	Typical Batch Method*	AES High-Density Method
Density of Rotifers	500 per ml	2,000 per ml
Tank Size	100 liters	100 liters
Algae Requirement per Tank	75 liters Fresh Algae	250 ml Algae Paste**
Total Rotifers per Tank	50 million	200 million
Tanks Needed for One-Week Feed	10–14 Tanks	1–2 Tanks
Estimated Equipment Cost	\$12,000–\$15,000	\$3,000–\$6,000

The PAES High Density Continuous Rotifer Production System

The user needs to provide an oxygen source (oxygen regulator and oxygen tank), air source, water source, temperature control, algae and rotifers. Pentair Aquatic Eco-Systems can also supply the rotifers, algae, heaters, chillers, air pumps and oxygen accessories separately. The 150-liter system has a footprint of approximately .75 m x 1.1 m. The 450-liter system has a footprint of approximately 1 m x 2.2 m. The 1,000-liter system has a footprint of approximately 2 m x 2.5 m.

Current production in these systems is ranging from 3,000–10,000+ rotifers per ml. Most users grow *Brachionus plicatilis*, but the system also works effectively for *B. rotundiformis*. The optimum temperature depends on the species: *B. plicatilis* requires 24–27°C and *B. rotundiformis* requires 27–30°C.

Temperature can be maintained by locating the system in a temperature-controlled room or by using heaters and chillers. Salinity is best maintained at 18–22 ppt and pH at 6.5–7.5, as the lower pH reduces ammonia toxicity. Harvest rates range from 25–70 percent per day with total recovery depending on the user. The water exchange is done through the harvest.

Once started, the system takes about two weeks to reach full production, so that two or more units are recommended to ensure continuous supply and provide redundancy. The system takes most technicians about one hour for daily maintenance, including counting and harvesting rotifers, refilling the feed and ammonia neutralizer reservoirs and cleaning. High-density rotifer culture systems reduce labor, water requirements and equipment costs. Keep in mind, however, that these systems have a learning curve. Transition time is necessary to make a successful system.

Water Quality Parameters

Temp: 28°C

Salinity: 18–22 ppt

pH: 6.5–7.5

DO: 5–10 ppm

Unbound Ammonia*: <1 ppm

Bound Ammonia*: <25 ppm

*Bound and unbound ammonia refer to the amounts of ammonia affected by ClorAm-X®.

All water quality parameters should be taken from the culture tank.

Samples can be centrifuged or filtered through 1 micron or less filter paper for the ammonia tests.

Ammonia Tests: Salicylate method for unbound ammonia and Nessler method for unbound ammonia and bound ammonia.

Protocol for Rotifer Counts

1. Use 200-ml glass beaker to obtain at least 100 ml of culture water from rotifer tank. The water should be taken from the surface, between the filter pads.
2. Mix sample by stirring water with 1-ml pipette. Do not create a current. Once the water is mixed, remove 1 ml and place in a clean Sedgwick rafter cell.
3. Examine the first sample for the following items before placing cover slip on slide and make notes as necessary. This information can help you determine whether or not the culture is healthy:
 - Do the majority of females have single or multiple eggs?
 - Rotifer swim speed—is it erratic, very slow, fast or normal?
 - The amount of detritus in sample shows how well filter is working.
 - Take note of any animal other than rotifers in sample (such as ciliates).
 - Large numbers of small rotifers and females w/single eggs.
4. Add one drop of prepared Lugol's solution to sample and place a cover slip on Sedgwick rafter cell. You can also use a chlorine or formalin solution. However, Lugol's solution actually tints the rotifers, which makes it easier to see them in the counting cell.
5. Start counting the sample from the upper left corner of the slide. Focus on no more than three rows at a time. This will help avoid confusion as to where you have counted before.*
6. Count the sample for the total number of rotifers.
7. Count the sample for the total number of eggs and determine the egg percentage.

8. Repeat steps 2 through 6 two more times. Samples should be uniform, so skip step 3.
9. Record all three sets of numbers.
10. Determine the averages of the rotifers/ml based on the 3 samples.

**Please note the following when doing counts:*

- Some dead rotifers will look like they have "spit up" their insides. DO count these rotifers since they have been killed by the Lugol's solution.
- Other dead rotifers will look like a shell with no visible insides near them. DO NOT count these—they were dead when the sample was taken.

Algae Calculations

1 bag of nanno paste (1,000 ml) will feed approximately 1 billion rotifers; therefore, 1 ml feeds 1 million rotifers. This is a rule of thumb that can be adapted to your particular needs.

Step 1: Determine total number of rotifers in culture tank after the harvest.

Step 2: Determine total number of rotifers needed/wanted for the next day.
Desired amount of rotifers should not exceed more than two times the current tank total.

Step 3: Calculate feed.

Algae Reservoir (in this case a 5-gallon drink cooler):

- Clean out daily.
- Add nanno paste to cooler and fill to 16 liters with salt water.
- Add 3 liters of crushed ice twice a day.
- Adjust air if needed (nanno paste should be rolling gently).

Algae can be stored in a compact refrigerator as well. You would only need to drill a hole through the side to accommodate the tubing for the pump and aeration.

Dosing Pump:

- Determine the total volume to be fed out as well as the volume that can be pumped through your chosen tubing. For example, 240 ml of paste solution can be fed out in 2.8 minutes using the 1/8" tubing.
- 2 minutes and 40 seconds on and 12 minutes off for the repeat cycle timer will feed out approximately 24 liters of paste solution in 24 hours.

ClorAm-X® Dosing

Please note that the amount of ClorAm-X® you will need to use is an estimate. The ammonia should be checked frequently in the beginning to make sure that the dosage is appropriate.

Step 1: Determine the amount of ClorAm-X® needed. For every 1 ml of nanno paste you will need approximately .07 g of ClorAm-X®.

For example, to feed 800 ml of nanno:

$$800 \text{ ml} \times .07 \text{ g} = 56 \text{ g of ClorAm-X}^\circ \text{ to feed out}$$

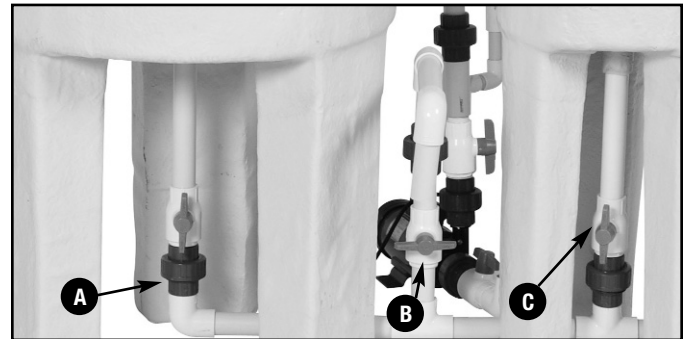
Step 2: Dissolve powder in a small amount of fresh water.

Step 3: Add this mixture to the algae paste container. It will be dosed to the system.

Rotifer Harvest

The system works best when there are at least two harvests per day. You can increase the number of harvests to more than two but it is not necessary unless your hatchery protocol requires it. The goal is to harvest 50% of the rotifers per day and rebound to the original numbers the following day. In the beginning, you will not need to harvest. In fact, unless the water quality is suffering, a harvest should not be attempted until the rotifer density is over 1,000 per ml, at which point the harvest should be small (10–25%). The system should be started at a minimum of 250 rotifers per ml. If it is started with densities below 100 per ml, a population crash is likely. It typically takes about 5 to 7 days to reach 1,000 per ml. This length of time it takes depends on how healthy the culture is and how experienced the culturist is.

In order to harvest, you should either purchase or make the appropriately sized harvest bag/apparatus. To begin harvesting, close valve C, remove the center standpipe from the culture tank and then open valve B. Plumbing out of valve B has been left to your discretion. The whole system can be elevated so that there is enough room to have a harvest bin. It is also best to have the system elevated to avoid the main pump coming in contact with standing water. After harvesting the required amount of rotifers, close valve B, replace the center standpipe in the culture tank and open valve C.



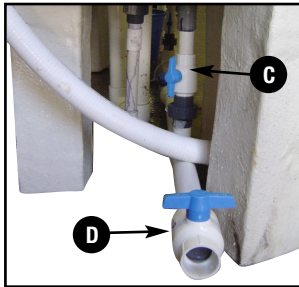
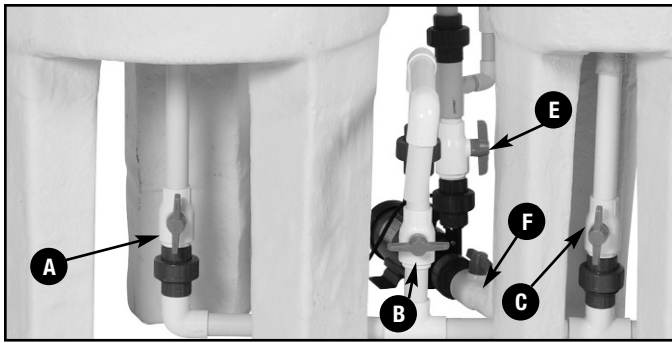
Cleaning the System

Filter Material

Clean two to three times a day using high-pressure fresh water from a hose. The system is shipped with enough filter material to make two sets of pads. Switch and sterilize the pads often.

Center Standpipe

- Clean twice a day.
- Bubble ring around the base must be removed and put back before and after cleaning.
- Harvest/drain valve A MUST be closed when the standpipe is out and REOPENED when it is back in place.



Protein Skimmer

- Clean weekly (clear portion on top) and monthly (main column).
- Close valve (E).
- Remove clear plastic top and arm.
- Use bristle brush to clean out center.
- When cleaning the column, open valve G on base so it may drain.
- When finished, reopen valve H to allow a light foam to develop.



Culture Tank

- Clean once a week.
- Make sure valves A and B are open while C is closed. At no time will you need to adjust the protein skimmer or pump valves (E and F).
- Remove filter material and center standpipe.
- Harvest the rotifer culture tank while the biofilter, water pump and protein skimmer continue to run. See harvest protocol on page 3.
- Flush culture in harvest bag with full strength seawater for 10–15 min.
- Clean tank, center standpipe, filter material and heater (if applicable).
- Change or clean air and oxygen diffusers.
- Fill tank with new salt water, add algae paste and restock tank with desired amount of rotifers.
- Make sure appropriate valves are reopened (labeled above).



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