

PROTOCOL DOCUMENT



Rotifer Cultures in Room D

The rotifer cultures are monitored and harvested daily.

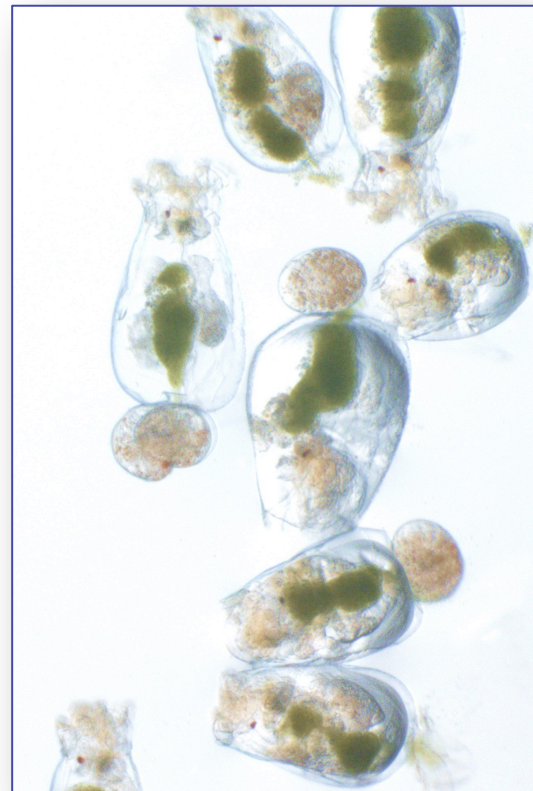
Rotifers are cultured using the **batch method**, where a given volume of water is inoculated with a rotifer starter culture, the rotifers are then allowed to reach a terminal density before being harvested and the culture is then restarted at regular intervals (3-5 days).

Parameters

- pH: 7-8.5
- Salinity: 15ppt
- Ammonia: ≤ 3 mg/L
- Temp: 25-28 °C
- Dissolved oxygen: >4 mg/l

Equipment

- 30L buckets
- Tropic Marin or Instant Ocean salt
- Microalgae
- Live Brachionus culture
- Airline and air diffusers
- Heaters
- Detritus strips
- RO water
- Rotifer sieve
- Ammonia blocker
- Measuring cylinder
- Beaker(s)
- Refractometer
- Cleaning Cloths
- Cut up filter mat
- Clicker counter
- 70% ethanol



Daily Routine

09:00

- Feed rotifers (30-50 ml/tank)

11:30

- Count up each rotifer tank to check density (using rotifer population worksheet)
- Determine how many fish tanks need rotifers (12:30 and 15:00 feedings) and aliquot rotifers to them accordingly (in 5ppt water)
- Harvest enough rotifer to feed the nursery tanks twice.
- Replace the traps for ciliates and impurities (2 detritus strips) for each bucket

Between 14:00 - 16:00

- Inoculate new bucket with the remainder of the harvested bucket
- Prepare the two buckets with 30L of fresh salt water (15ppt) for the next day
- Feed rotifers (30-50 ml/tank)

Population check

- Check the population daily
- Use a microscope to check the density
- From each bucket collect approximately 7ml of the culture in 15ml falcon tubes
 - Collect the sample from a few different areas of the surface of the culture
- Take 1 ml of each sample and place on the gridded petri dish
- Add 1 or 2 drops of iodine (Lugols solution) from the iodine falcon tube
- Using the clicker counter measure the following parameters:
 - Total number of rotifers per ml
 - Fertility as the percentage of females carrying eggs (total number of females carrying eggs / the total number of rotifers)
- If the total population contains less than 20% of fertilized females, the system may be crashing
- Check for ciliates

Harvesting

- Harvest daily
- Harvest from each culture in a rotation
- When possible use only a single culture per day (preferably the oldest one)
- Mark the used culture on the rotifer population worksheet

Determine amount required by counting the tanks in the nursery and juveniles

$\frac{\text{Number of tanks (total)} \times 25,000}{\text{Rotifers per ml (from your counting)}} = \text{ml of rotifer culture to harvest}$
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Example:

$\frac{40 \times 25,000}{200} = 2,500\text{ml or } 2.5\text{L}$

- Put this in the 'amount to harvest' box on the sheet
- Additional rotifer may be needed for the other nursery and the killifish

The harvested rotifer then needs to be concentrated for feeding

- Each tank is fed 6ml of concentrated rotifer
- Number of tanks x 12 = ml of concentrate needed for two feeds
- Put this in the 'amount to feed' box on the rotifer population worksheet

To Harvest

- Switch off the heater
- Prepare the required amount of 5ppt water using a measuring cylinder
- Sieve rotifer through the green sieve
 - Be careful not to disturb the water in the culture too much
 - If this is not done carefully detritus that has settled to the bottom will foul the culture
- Rinse the rotifer from the sieve into a beaker using 5ppt water to the amount needed
- Once concentrated feed 6mls to each tank
- After feeding add 1ml of algae per litre of concentrate that remains in the beaker

Making a new culture (Inoculation)

- After harvesting remove the air diffuser and heater and place in a clean salt bucket
- Allow culture to settle for approximately 5 minutes
- During this time clean the air diffuser and heater in a sink then wash with 70% ethanol
- Transfer the air diffuser to the new bucket of 15ppt water ready from the day before (it should be bubbling gently in the centre of the bucket)
- Add the heater and switch on
- Add 30 mL of microalgae (previously diluted with 15 ppt water)
- Add the traps for ciliates and impurities (2 detritus strips)
- Inoculate the bucket with rotifer from the culture that has been left to settle
 - Try to achieve an initial density of approximately 100 rotifer per ml
- Clean the emptied culture bucket in a sink using tap water and cut up filter pad using 70% ethanol

To harvest the old culture for inoculation

- With a beaker **carefully** remove approximately 2L of culture at a time
- Gently pour through the green sieve over an empty salt bucket
- Repeat twice more (6L of culture total)
- Rinse the rotifer that is in the sieve using 15ppt water (from a pre-mixed bucket) using a clean beaker
- Transfer the rotifer in the sieve to the new culture bucket
- Repeat these steps making sure that the dirty water bucket gets emptied before it is too heavy

Do not let the rotifer dry out in the sieve

If this is not done carefully detritus that has settled to the bottom will foul the culture, wait until it settles out again then continue to harvest

Do not harvest the bottom 10cm of the culture

Making Salt Water

Add the required amount of salt to the bucket before adding the water, then add RO water to the 30 L mark.

To get to the correct

$\frac{(41.8 \times \text{ppt})}{35} =$	Salt needed (grams per litre)
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 salinity:

Two salinities are required, check them with a refractometer:

⇒ **15 ppt** - 17.91g/L or 537g in 30L

⇒ **5 ppt** - 5.97g/L

Add one heaped tub (kept in the salt bucket) to a 32l bucket; fill the bucket with R.O. (or system water if not available) to the lip in the bucket, then position under the rack. Add an air diffuser to mix. This will give a mix of approximately 15ppt. Test with a refractometer and adjust as necessary.

Feeding

To Prepare Algae

- Defrost the concentrated Nannochloropsis paste in the fridge overnight
- Once defrosted aliquot algae in 40ml portions into 60ml falcon tubes and re-freeze

For Feeding

- Defrost the required amount of algae in the fridge overnight
- Suspend microalgae concentrate in 15ppt salt water
- Mix 1 part algae to 3 parts salt water
 - If using only Nannochloropsis use 150ml of algae and 450ml of 15ppt water
 - If using Nannochloropsis and Isochrysis - use 115ml Nanno and 75ml Iso to 410ml of 15ppt water

To Feed

- Pour algae mixture into rotifer culture until the culture is opaque
- Feed the rotifer cultures once the culture becomes translucent

DO NOT allow the algae to warm up. It must be kept chilled at all times or it will go off

Water Quality/Environmental parameters

The water quality must be monitored daily (record the results in the rotifer population worksheet).

Test For

- pH
- Ammonia
- Oxygen
- Temperature
- Salinity

Note: Aeration must be in the centre of the bucket and monitored daily. Change the air diffuser as necessary.

Useful Figures and Formulae

Salt/Salinity

To increase salinity:

$$((\text{Required ppt} - \text{Measured ppt}) \times \text{Volume in litres}) \times 1.2 = \text{Grams of salt to add}$$

- 12g/l \approx 10ppt
- 1.2g/l \approx 1ppt

To decrease salinity:

$$\frac{\text{Volume in litres}}{\text{Measured ppt}} \times \text{Reduction needed in ppt} = \text{Litres to remove}$$

- Then refill to original volume with reverse osmosis or system water

Ammonia Blocker (Aquapure)

- $(32 \times \text{ammonia concentration} \times \text{volume}) / 1000 = \text{grams to add}$
- 0.96g in 30 litres decreases ammonia by 1mg/l
- 32mg in 1 litre decreases ammonia by 1mg/l