

Zebrafish lab protocol from IGIB
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Paramecia culture protocol

Materials required:

Beaker	2 L
System Water	1200ml
Yeast Powder	0.6g/1.5L
Wheat Beads	40-45/1.5L
Starter culture	300ml/1.5L
Total Volume	1500ml

Method

1. Take 400ml of system water (RO water + salt) in a beaker (1L beaker).
2. Add 40-45 number of wheat seeds to the above beaker and bring to boil for 2-3 minutes in a microwave oven.
3. To this immediately add 0.6g of finely grinded yeast (get from local market) powder and mixed gently.
4. Cool the above solution to room temperature.
5. Adjusted volume to 1200ml by adding system water (RO water + salt).
6. Finally add 300ml of the filtered paramecia culture (inoculums) to the above solution. Mix gently.
7. The culture is kept for growing.
8. A total of 7 cultures were maintained per week.
9. Every 7 days old culture is used for inoculation and feeding.

Note:

1. Culture used for inoculation should be rich/pure. (The culture should be checked for the density and other contamination under a microscope).
2. Autoclaved glassware's should be used.
3. Culture with any sign of fungal growth or other species should be discarded immediately by autoclaving.
4. Only system water should be used.
5. **Algal growth (greenish in appearance) has found to be normal for the culture, hence has no threat.**

Embryo feeding protocol

*Pure Paramecia culture which are 7 days old and showing high density are used for feeding the embryos as follows.

Dpf	Frequency of feeding	Quantity of feeding
5 – 20	One time paramecia	Minimum 20ml/20 embryos. For additional embryos use culture in multiples of 5ml (e.g. for 30 embryos use 30ml culture)
20 – 30	Morning – Fresh Brine shrimp. (Diluted culture only). Evening - Paramecia culture	Morning - Approx one drop of diluted culture/embryo. (Do not overfeed) Evening - Minimum 20ml/20 embryos. For additional embryos use culture in multiples of 5ml (e.g. for 30 embryos use 30ml culture)
30 - 60	Morning & evening (brine shrimp)	Approx one-two drop of diluted culture/embryo. (Do not overfeed)

Adult Fish feed preparation protocol

Materials required:

Brine shrimp egg.	1.8g/L
Feed culture tank	15L
Aerator	2 each/tank
System water	Adjust to 12 L
Salt	35g/L
Total volume	12L

Methods

1. The tank is cleaned first with tap water using the softer side of the scrubber followed by rinsing with system water.
2. 8-10 liter of system water (RO water) is filled into the tank.
3. 420g (35X12) of salt is added (35g/L) and allowed to dissolve.
4. The aerator is adjusted for proper aeration.
5. The volume is adjusted to 12 L.
6. Finally the Brine shrimp egg is added after the salt is completely dissolved.
7. The brine shrimps take 48 hours to hatch.
8. At the end of 48hrs the hatched shrimps (orange in color) are ready for feeding.

Note:

1. The tank should be cleaned very nicely using the softer side of the scrubber.
2. Aerator should be adjusted properly.
3. The eggs/cysts shouldn't be allowed to adhere on the wall of tank.
4. Before adding the brine shrimp egg the salt should be allowed to dissolve completely.
5. The tank should be labeled correctly for easy identification.
6. The feed should be washed thoroughly with system water to remove salt content before collecting on the feed bottle.

Feed Collection Protocol

Material required

Beaker----- (2L)
Beaker----- (1L & 500ml)
Filter net
System water----- variable

Method

1. At the end of 48hrs the brine shrimp are properly hatched and are ready to be collected.
2. The hatched brine shrimps are allowed to be collected on the filter net.
3. Once the brine shrimps are collected on the filter net, system water is used to wash it (salt should be completely washed).
4. Washed brine shrimps are collected on the 2L glass beaker and diluted with system water.
5. The beaker is allowed to stand for 8-10 min to allow the shrimps to settle down. Any residual cyst comes up and will float on the surface of water.
6. The upper layer-containing cyst is discarded and rest of the solution used for feeding.

Note:

1. The unhatched eggs should be avoided while collecting the feed.
2. The salt concentration should be properly diluted by washing with system water.

Feeding Protocol

1. Once the feed is collected into the beaker, it is diluted and then transferred to the feed bottle.
2. Freshly collected brine shrimp is used for feeding.
3. Sterile yellow tips with fine cut on top should be used every time to avoid contamination.
4. Feeding is done thrice a day.

Note:

1. The concentration of feed should be adjusted properly.
2. Feed should be given such that the fishes can finish feeding on it within 5min.
3. Special care should be taken while feeding single fish.
4. Cyst/unhatched eggs should be avoided.
5. Feeding is done thrice in a day for the evening feeding the collected feed is preserved in 4⁰C.

Breeding setup protocol

Material required:

Beaker	2 L
Bucket	25 L
System water	600 ml/breeding tank
Breeding tank/chamber	variable
Fishes	one pair/ tank

Methods:

1. Clean-washed breeding tanks/chambers are used for breeding setup.
2. Adequate water is poured into each tank.
3. A pairs of healthy male and female fishes are put into the tank.
4. Divider is placed to separate the male from the female fish.
5. Male is separated from the female for whole night.
6. Next the divider has to be removed in the morning exactly 5 minute before the light gets on.
7. The tanks/chambers should be checked for embryos after 20 minute the divider gets removed.

Note:

1. There should be a dark period of 10hrs as the fish behaves photo-periodically in there breeding.
2. The fish kept for breeding shouldn't be disturbed.
3. The breeding tanks used should be properly washed and autoclaved each week.
4. Night feeding should be avoided for the breeding fishes.*

Embryo collection protocols

Material required –

1. Fine net.
5. Embryo water.
6. Petri plate.
7. Fish net.
8. Pasteur's pipette

Step 1. Each tank is checked for embryos.

Step 2. Tank with embryos is kept separately.

Step 3. Breeding pairs are removed and returned to the system/ holding tank.

Step 4. Embryos are collected on a fine net by pouring into the net.

Step 4. The net is reversed and embryo water is poured over it to collect the embryos on a petriplate.

Step 5. The net is checked for any embryo.

Step 6. The embryos are counted, cleaned using pipette and divided into max of 100 embryos per Petri plate.

Step 7. 3 drops of methylene blue (0.03%) is added per Petri plate to prevent any fungal growth.

Step 8. All the plates are stored at 29°-30°C incubator.

- Note .
1. Breeding pairs should be removed carefully.
 2. Fresh embryo water should be used every time.
 3. Special attention should be paid while separating the breeding pairs,

Embryo rearing protocol

Material required

1. Embryo rearing tank
2. Embryo water
3. Embryo Feed (good quality paramecium)
4. Labeling tape
5. Water proof marker

Procedure:

1. The embryos are put for growing on 5th dpf.
2. 5 days old embryos are carefully put into the embryo rearing tank filled with adequate amount of embryo water (RO water + salt)
3. The label containing all the details for each tank is kept ready and pasted to the designated tanks.

- Note:
1. 2.9 L tank ----- 20 -30 embryos
 2. 9L tank ----- 100 embryos.
 3. Only embryo water should be strictly used.
 4. Water level should be proper.
 5. Water should be changed daily with fresh embryo water.

* For feeding the embryos please refer to feeding protocol.