

Larval Rearing and Seed Production of Silver Pompano, *Trachinotus blochii*

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It is generally accepted that the larvae stage of marine fish production is the major 'bottle neck' in the development of mariculture. Rearing fish larvae is a challenging part of fish culture because larvae are very sensitive to water quality, have high nutritional requirements and require the use of live feeds. A continuous supply of live or dry feeds and a controlled environment, i.e. temperature, filtration, photoperiod, oxygen and pH, are essential for any experimental or commercial system. For marine fish larvae live zooplankton prey has to be offered because the survival and performance of the larvae is poor on formulated diets. Furthermore, it is common practice to add live or inactivated microalgae to the rearing systems as this has shown to be beneficial for the larvae.

Zooplankton culture

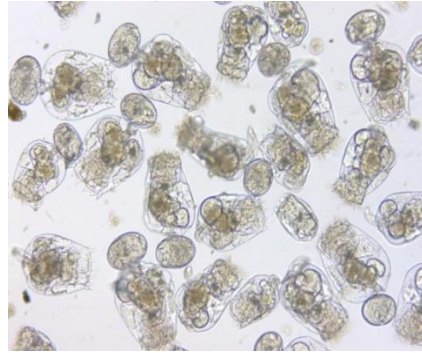
Rotifer

Common zooplankton live feed species used in hatchery rearing of fish larvae are rotifers, artemia and copepods.

Rotifer belongs to the genus *Brachionus* and is commonly used in Aquaculture. For breeding marine fishes, the rotifer *Brachionus plicatilis* is the most widely used live feed that can be used in early larval stages. Rotifers can be grown in mass quantities for aquaculture because of their rapid reproduction, amenability for high-density culture, tolerance to the temperature and salinity, and acceptability by larval fishes. Two main strains are used in hatcheries 1.Small strain (S-type with average lorica length of 130 μ) 2. Large type (L-type with 240). Both strains show different temperature and salinity tolerances. Choice of rotifer differs in accordance to the mouth size of the larvae. Rotifers can be cultured in small glass tanks of 50 L capacity to 10 t tanks. To get good yield, water quality parameters should be at the optimum level; temperature at 25-28°C, pH at 7.5-8.5, salinity in a 20-30 ppt range, less than 1 mg/l free ammonia (NH₃). Stock culture should be maintained separately. Inoculums should have 20% fertility rate with a density of 150-200/ml. Rotifers can be fed with different micro algal species such as *Nannochloropsis* spp., *Isochrysis galbana*. Rotifers are usually mass cultured in 2 -5 ton FRP tanks at the salinity rate of 28-30 ppt and fed with concentrated algae of *Nannochloropsis* sp. For mass production of rotifers, baker's yeast is used as supplement and later harvested rotifers were enriched with algal based enrichment medium (ORI green -Skretting). Aerating the rotifer culture is essential to provide oxygen, to keep rotifers and food cells in suspension and to optimize tank cleanliness.



Mass culture of rotifer in large tanks



Rotifer *Brachionus plicatilis*

Though rotifers are not nutritionally rich when compared to the copepods its nutritional composition can be adjusted in a relatively short time by enrichment method. The microalgae can be cultured on site: If algal culture facility is not available rotifers can also be fed with algal paste or commercial rotifer feeds.

Artemia

Brine shrimp artemia is the second most widely used live feed after rotifer. Availability, storage as cyst, ease of hatching, and nutritionally rich naupliar (430 μm) stages and adaptability to various culture conditions makes this species one of the most important live feeds. Many commercial brands artemia cysts are available in the market. Hatching rate and number of cyst/gram and the size of the nauplii are the parameters that characterize the artemia cyst quality. Artemia cysts were kept for hatching after decapsulation process that involves treatment in a chlorine solution, washing and deactivation of the residual chlorine. The hydration, a necessary step as the complete removal of the chorion. Artemia should be harvested (22 h at 28°C) at the energy-rich instar I larval stage, just after hatching. This can be fed to the larvae after proper washing. It can also be enriched with enrichment medium after 12 HPH.



Artemia nauplii



Artemia metanauplii

Egg collection, packing and transportation

After hormonal injection (both doses), frequent observation of brood stock tank is essential to find out the exact time of spawning. This can be done by collecting a beaker of water and observing for eggs without disturbing the brooders. Lower salinities will decrease the buoyancy of eggs hence the brood stocks should be maintained in 35 ppt seawater during the spawning. After spawning floating eggs are collected using 250-300 μ mesh by sieving and remaining eggs are collected from the overflow conduit of the egg collection chamber tied with fine mesh cloth. Egg collection nets must be chlorinated and washed properly after every use.

Only good quality floating and transparent eggs are used for larval rearing and unfertilized egg which is opaque and settles down at the bottom are discarded. To differentiate the fertilized eggs from unfertilized ones: all the collected eggs were transferred to 500 L hdpe tank, and it is allowed to settle for 10-15 minutes without aeration. Fertilized eggs will float and form thick layer on the surface of the tank whereas poor quality eggs will float in the deeper layers of water whereas dead opaque eggs will settle at the bottom. Only floating eggs in the surface layer can be segregated and transferred to another temporary container for assessing the quality of eggs.

Quality assessment of the fertilized eggs and counting

Sub samples of eggs collected from the temporary container was observed under microscope with 4x or 10 x magnification to assess the quality and viability. Good quality viable eggs should possess the following characteristics such as transparency, proper shape, free of dead eggs, ciliates, parasites and number of oil globules etc.

Eggs which has more than 80% fertility and hatching rate are preferred for larval rearing after treating with 20ppm iodophor for about 5min.

Fertilized eggs of silver pompano are spherical, pelagic, transparent and non adhesive with single oil globule and it measured 0.9-1.0 mm in diameter. Eggs were collected from the brood stock tank at the optic vesicle stage of embryonic development to avoid high mortality due to physical damage of eggs. Collected fertilized eggs are transferred to known volume of seawater, after through mixing of water 2-3 sub samples (5-10 ml) are taken and eggs are counted and the average is taken and multiplied with the total volume of eggs in seawater.

After counting, the egg can also be transferred to hatcheries at far away locations by air or by train. Counted eggs are transferred to 32"x16.5 "polythene bags with 2/3 of oxygen and 1/3 of water. Stocking density of eggs per bag vary with travel distance and hours of transportation to reach the destination.



Fertilized eggs silver pompano



optic vesicle stage

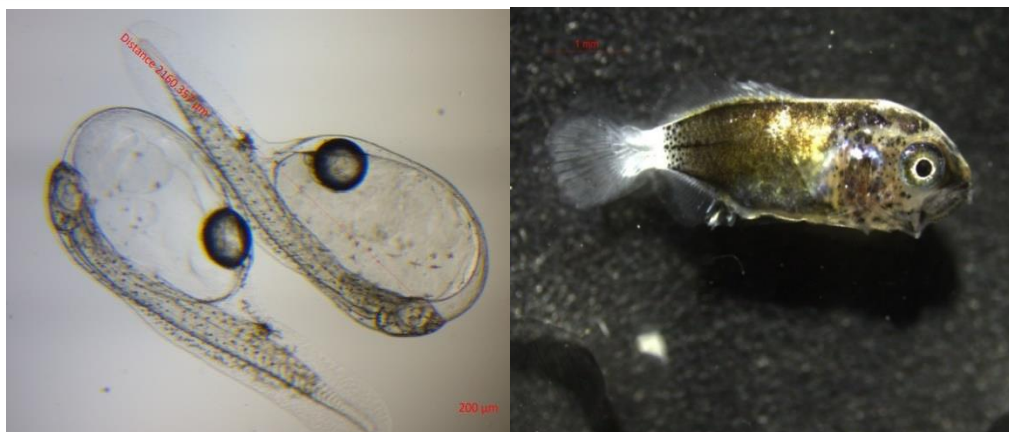
Disinfection of larval rearing system

Before stocking of larvae in rearing tanks entire larval rearing system has to be disinfected with 500 ppm chlorine (filters, tanks and aeration system etc)

Larval rearing seed production

Circular tanks (FRP) of blue color with 3 to 5 ton capacity are used for larval rearing with approximately of 1.2 m of depth. Commercial brand (Sano life MIC powder, Inve Aquaculture) probiotics was mixed with seawater @ 2g/ton of seawater is also added to the tank an alternate days till 5 dph. Pretreated filtered seawater of 35 ppt passed through UV filter is used for the larval rearing. Eggs are stocked at the density of 10 nos/L in LRT with green water medium. The green water culture system (35 ppt) consists of algae: *Nanochloropsis salina*, *Isochrysis galbana* and *Chaetoceros calcitrans* and rotifer, *Brachionus plicatilis*. Mild aeration was given to the LRT (1 air stone/ton of water).

Hatching of larvae begin after 16-18 hrs of spawning at the water temperature of $28\pm 1^{\circ}\text{C}$. on 1st dph, dead eggs, egg cases from tank is removed by siphoning out the bottom water. Newly hatched larvae measured of 1.9 to 2.0 mm in total length and the yolk sac length was 150 μ . Newly hatched larvae had unpigmented eyes and closed mouth. At 2nd dph (days of post hatch) larvae will have reduced oil globule, completely absorbed yolk sac, pigmented eyes and fully functional mouth. On 3rd dph larvae measured between 2.9 and 3.2 mm. Rotifer is as used as first feed and in later stages larvae are fed with artemia and the larvae are slowly weaned to artificial feed.



Newly hatched larva

10 dph larva

L-type (Large) *Brachionus plicatilis* rotifers are added to the larval rearing tanks from 1st dph to till 10 dph at the density of 6-10nos/ml. Size of the rotifers ranged from 166-210 μ . Bottom sediments from the larval rearing tanks are usually removed on 1, 3, 6, and 10 and 16 dph.

Rotifers were enriched with commercial algal based enrichment medium (Ori green skretting) for about two hours or Normal algal enrichment (combination of algae) before feeding to the larvae. Dosage and time for enrichment varies with each enrichment medium and follow the instructions given in the pack. To maintain the rotifer density: tanks were checked periodically, and rotifers are added if necessary.

Newly hatched Artemia nauplii are added to the LRT tanks from 9 dph onwards along with rotifers as additional live feed at a stocking density 2-3/ ml.(when larvae are introduced to the new feed: new feed has to be fed to the larvae in empty stomach after that the regular diet has to be given). The total length of the pompano larvae was 6.3 ± 0.2 mm. Co- feeding of rotifer with artemia (slowly reduce the number of rotifer and increase the artemia nauplii concentration) continued up to 12 dph till the larvae completely weaned to artemia diet. Artemia were enriched for 22 to 24 hours (enriched artemia) was fed to the larvae from 12 dph onwards and it was continued till 18 dph.

At the same time (from 18 dph) larvae were also fed with artificial micro diet of 200 μ size and slowly reduced the artemia density in the LRT. The micro diet was continued till the larvae completely weaned to artificial diet. By 20dph the larval length was 9.09 ± 1.3 mm. Larvae were slowly trained with pellet feed in starved stomach later with regular feed.

Pompano larvae are black in color till 6 dph later transforms in to white between 6 and 8 dph. Again it becomes black by 9 dph and it continues until complete transformation of larvae or metamorphosis ends: which begins by 18 dph and it is completed by 28 dph. During that period larvae develops scales on the body and becomes juvenile and the total length of the metamorphosed larvae is 1.0-1.5 cm. Water management and Larval rearing protocol of Silver pompano, *Trachinotus blochii* is summarized in the following table.

	0	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	25	30	35	40	45	50	
<i>Nanochloropsis salina</i> + <i>I.galbana</i> + <i>C. calcitrans</i>	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green									
large rotifer (4-10 /ml)		Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow												
Artemia nauplii (3-5/ml)										Brown	Brown	Brown											
Artemia enriched (3-5/ml)												Light Brown	Light Brown	Light Brown	Light Brown								
Artificial feed (200 μ)															Orange	Orange	Orange						
Artificial feed(200-400μ)																		Pink	Pink				
Artificial feed(300-500μ)																			Pink	Pink			
Artificial feed(500-800μ)																			Dark Red	Dark Red	Dark Red		
In situ biofilter															Purple	Purple	Purple	Purple	Purple	Purple			
Bottom siphoning		Red		Red			Red				Red												
WATER MANAGEMENT																							
water exchange 10%		Blue																					
30%			Blue			Blue					Blue												
50%													Blue										
Running water/100% exchange																	Blue	Blue	Blue	Blue			
Running water /200% exchange																					Blue	Blue	Blue

Fingerling production.

Metamorphosed larvae of 1.5 cm size are transferred to D- shaped tank of 6 ton capacity RAS @stocking density of 25000-30,000 and the post larvae are fed with artificial diet of 300/500 μ till 40 dph and after 40 dph juveniles are fed with size 800 μ pellet feed. Larvae are fed adlibitum level at 7.00 am, 12.00 am, 4.00 pm and 6.00 pm. Juveniles attain 5- 7 cm after 45-60 days of rearing.
