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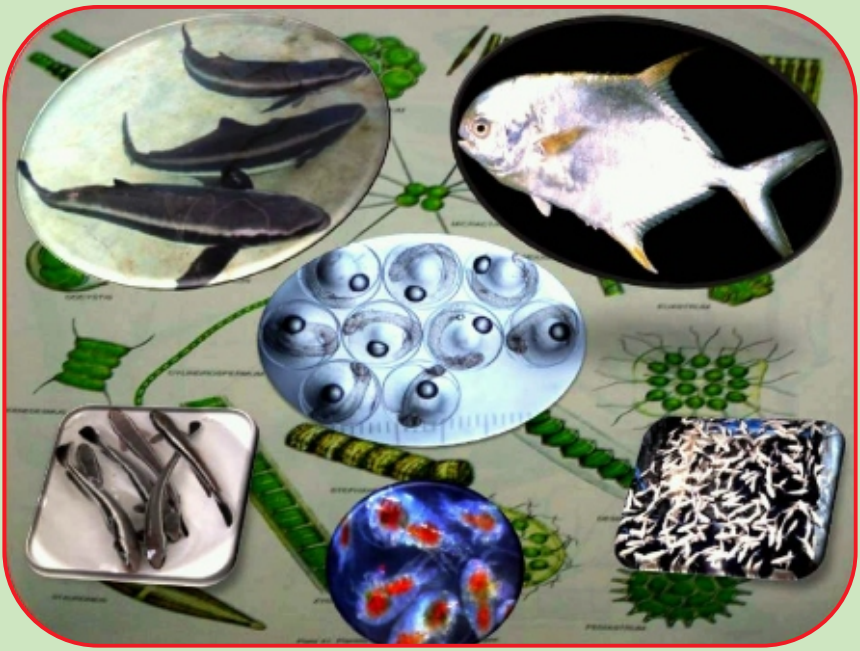
Practical Hand Book on
Seed Production of Cobia and Silver Pompano



Mandapam Regional Centre of CMFRI
Mandapam Camp - 623 520
Tamil Nadu – 623 520

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Central Marine Fisheries Research Institute

Practical Hand Book on
Seed Production of Cobia and Silver Pompano

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PREFACE

Mariculture – *the farming and husbandry of marine plants and animals of commercial importance,* is an untapped sector of sea food production in India. Mariculture has been contributing substantially to the global fish production in recent years. It accounted for about 29.2 per cent of world aquaculture production by value. It is a fact that India is still in infancy in mariculture production in the global scenario. Since mariculture is the only hope for increasing seafood production in the coming years, the research and development in this sector is of paramount importance. In fact, such thrust was not given in India and hence, it was felt necessary to develop viable seed production and farming technologies to develop mariculture. The R&D efforts in this direction yielded commendable results within a few years and Mandapam Regional Centre of ICAR CMFRI succeeded in captive breeding and seed production of two high value marine fishes namely, Cobia, *Rachycentron canadum* and Silver pompano, *Trachinotus blochii* for the first time in the country. Subsequently many trials on seed production and farming were conducted to develop techno-economically viable methodologies.

Cobia and silver pompano are two marine finfish species with very high potential for aquaculture in India. Fast growth rate, adaptability for captive breeding, lower cost of production, good meat quality and high market demand are some of the attributes that make them excellent species for aquaculture. Broodstock development of cobia was initiated at the Mandapam Regional Centre of Central Marine Fisheries Research Institute in sea cages during 2008 and the first successful induced breeding and seed production was achieved in March 2010.

Subsequently CMFRI has successfully conducted the sea cage farming demonstration of cobia and the technology is being adopted by many fishermen groups in different coastal states. Results showed that the fish can attain an average weight of 1.0 kg in 4 months and 2.5 - 3.0 kg in 6- 7 months of farming period. Cobia attained an average weight of 7.0

kg to 8.0 kg within one year. These results point out the possibility of developing a lucrative cobia aquaculture enterprise in the country.

The silver pompano, another candidate species for mariculture is able to acclimatize and grow well even at a lower salinity as low as 8 ppt and hence is suitable for farming in the vast low saline waters of our country, besides its potential for sea cage farming. At Mandapam Regional Centre of CMFRI, successful broodstock development, induction of spawning and fingering production of silver pompano was achieved during July 2011 for the first time in India. Subsequently farming trials were conducted and fishermen groups and entrepreneurs are adopting the technology in different coastal states.

There has been an increasing demand for the seed of cobia and silver pompano from the coastal fishermen/farmers of the country. Realising the necessity of seed production, the NFDB, DADF, Govt of India had sanctioned 2 projects for ICAR CMFRI to enhance the seed production capacity of cobia and pompano so as to ensure steady supply of seed for the mariculture activities of the country apart from capacity building of the coastal fisherfolk through training programmes. In this context, it was felt necessary to prepare a practical handbook on seed production of cobia and silver pompano. I hope that the publication will be of help to the fisheries developmental agencies, researchers, hatchery technicians, entrepreneurs and fisherfolk.

Mandapam Camp

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INTRODUCTION

In recent years, mariculture has been growing rapidly on a global basis especially with the development and expansion of sea cage farming. On a global basis, a rapid growth in marine finfish culture is noted. It has increased at an annual average growth rate of 9.3% from 1990 to 2010. Salmonids, amberjacks, sea breams, sea basses, croakers, groupers, drums, mullets, turbot, other flatfishes, snappers, cobia, pompano, cods, puffers and tunas are the major groups which are maricultured. One of the major reasons for the growth of sea cage farming is the availability of breeding techniques that can produce sufficient quantity of seeds of different high value marine finfish. Many countries in the Asia-Pacific Region like Australia, China, Japan, Taiwan, Philippines, Indonesia, Thailand, Malaysia and Vietnam have made substantial progress in the development of commercial level seed production technologies of high value finfish suitable for sea farming. But even in these countries, seed stock supply is one of the vital issues for further expansion of mariculture. Currently massive efforts are being taken by the CMFRI for the demonstration of sea cage farming at different parts of our coast and commendable success has been obtained at many places.

Present status of marine finfish farming in Asia-Pacific Region

A large number of finfish species are farmed in cages and yet there is a significant reliance on wild caught young ones for farming of some groups such as groupers. The main species farmed in brackish water are the Barramundi or Asian sea bass (*Lates calcarifer*) and the Milk fish (*Chanos chanos*). Global production has been relatively constant over the past ten years at around 20,000 – 26,000 tonnes per annum, although production has decreased in Asia and increased in Australia during this period. In inshore marine cage farming, the major farmed species

include *Seriola* spp., snappers (*Lutjanus* spp.), groupers (*Epinephelus* spp.) and cobia (*Rachycentron canadum*).

The Japanese amberjack *Seriola quinqueradiata* contributes up to 17% of marine finfish production in Asia, with a production of about 160,000 tonnes annually. Nearly all this production comes from Japan, where production levels have been relatively stable at 140,000 – 170,000 tonnes per annum since 1980s. Other carangids that are becoming popular for culture are the snub-nosed pompano *Trachinotus blochii* and the silver pomfret *Pampus argenteus.* Nevertheless, seabreams are the mainstay of Asian finfish mariculture production, and a range of species are currently cultured.

Grouper culture has been expanding rapidly in Asia, driven by high prices in live fish markets of Hong Kong and China. Since grouper farming is mainly dependent on wild collected seed, the decreasing availability of wild seeds due to overfishing is a major constraint for the expansion of grouper culture.

Southern blue fin tuna (*Thunnus maccoyii*) is cultured in Australia using wild caught juveniles. Although production of this species is relatively less (3500-4000 tonnes per annum), it brings very high prices in the Japanese market and thus supports a highly lucrative local industry in South Australia. The 2003 production of 3500 tonnes was valued at US$ 65 million.

Cobia(*Rachycentron canadum*) is a species of much interest for tropical marine finfish aquaculture. Most production currently comes from China and Taiwan Province of China and it was around 20,000 tonnes in 2003. Production of this fast growing species is set to expand rapidly in Asia. Cobia has become a global commodity, in the same way that salmon has become a global commodity in temperate aquaculture.

Milkfish (*Chanos chanos*) is traditionally cultured in

Phillippines. Indonesia is a major producer of seed, much of this coming from small-scale hatcheries. Milkfish culture is also practised in some Pacific Islands *viz.* Kiribati,Nauru, Palau and the Cook Islands. Although most milkfish culture is undertaken in brackishwater ponds, there is increasing production from intensive mariculture cages.

Indian scenario

In India much research attention was not given for developing seed production methods for high value finfishes suited for sea farming. At present we have commercial seed production of only one marine finfish – sea bass (*Lates calcarifer*). Here also private entrepreneurship has not yet been developed. The vital issue for the expansion and commercialization of sea farming in India is the availability of suitable seeds of high value finfishes .Unless an intensified research and development effort is made to the development of commercial level seed production technologies, sea farming cannot emerge as a significant seafood production sector in the country.

The cage farming demonstrations conducted by CMFRI in the recent past have generated a lot of interest in the farming of marine fin fish among fish farmers and entrepreneurs. However, the lack of commercial scale availability of hatchery produced seed is the major bottleneck for any large scale venture of marine finfish farming. The availability of seeds from wild is often unpredictable and hence farming based on wild collection of seeds may not be a sustainable venture. Hence the development and standardization of seed production techniques for a few species belonging to groupers, pompano, snappers, breams and cobia should receive research priority. It is felt that the development of commercial hatcheries for ready supply of seeds is the primary step for the development and expansion of marine finfish farming in India.

Cobia and silver pompano – Two promising species for Indian mariculture

Cobia (*Rachycentron canadum*) and silver pompano (*Trachinotus blochii*) are two marine finfish species with very high potential for aquaculture in India. Fast growth rate, adaptability for captive breeding, lowest cost of production, good meat quality and high market demand especially for *sashimi* industry are some of the attributes that makes cobia an excellent species for aquaculture. In recent years the seed production and farming of cobia is rapidly gaining momentum in many Asian countries. Envisaging the prospects of cobia farming in India, broodstock development was initiated at the Mandapam Regional Centre of Central Marine Fisheries Research Institute in sea cages during 2008 and the first successful induced breeding and seed production was achieved in March – April 2010. Trials on sea cage farming carried out at Mandapam showed that the fishes attained an average weight of 2.5 kg in six months and 7.3 kg in twelve months. The species can be grown in low salinity and experiments revealed that upto 15ppt the growth and survival is comparable to that in seawater. These results point out the possibility of developing a lucrative cobia aquaculture enterprise in the country. However, standardization of technologies for seed production and farming of cobia to suit our environmental conditions have to be further pursued on a priority basis so that India can also emerge as a contributor for cobia production in the near future. Similarly among the many high value marine tropical finfish that could be farmed in India, the silver pompano is also one of the topmost, mainly due to its fast growth rate, good meat quality and high market demand. The species is able to acclimatize and grow well even at a lower salinity of about 10 ppt and hence is suitable for farming in the vast low saline waters of our country besides its potential for sea cage farming. At Mandapam Regional Centre of CMFRI, successful broodstock

development, induction of spawning and fingerling production of silver pompano was achieved during July 2011 for the first time in India. Subsequently few more seed production experiments were also done successfully. A pond farming demenstration of silver pompano at Anthervathi, East Gothavari District, Andhra Pradesh from hatchery produced seeds showed that in eight months period fishes have grown to 450 - 550 gm with an average survival rate of 95%. This can be considered as a milestone towards the development of pompano aquaculture in the country. The current achievements in cobia and pompano can be considered as the first step towards the aquaculture development of the two species. The establishment of biosecure broodstock centres, standardisation of breeding, larviculture and nursery rearing protocols and farming demonstrations in pond and sea cages are the steps to the way forward. Hence it is required to invest and establish infrastructure for the different phases from seed to product development *viz.* (i) Required broodstock facility for the production of viable fertilized eggs throughout the year (ii) hatchery facility for meeting the seed requirements (iii) grow out facilities and (iv) product processing and distribution system. It is felt that both cobia and pompano are potential aquaculture giants having vast domestic and global business prospects.

The major steps involved in cobia and pompano breeding and seed production are the following:-

1. *Broodstock collection*
2. *Transportation*
3. *Quarantine*
4. *Broodstock development*
5. *PIT tagging*
6. *Cannulation*

7. *Induction of spawning*
8. *Egg collection*
9. *Incubation*
10. *Larval feeding*
11. *Grading*
12. *Fingerling production*
13. *Nursery rearing*
14. *Micro-algal culture*
15. *Green water technique*
16. *Rotifer culture*
17. *Artemia hatching*
18. *Copepod culture*
19. *Nutritional enrichment of live feeds*

Broodstock

Broodstock Collection and handling

Broodstock fish are generally collected from the wild and are conditioned and matured in captivity. The main selection criteria to identify suitable adult fish as broodstock fishes are size, age (for those collected from grow-out farms) and appearance. The following are the details of the selection criteria:-

- *body shape, age and colour,*
- *absence of deformities,*
- *absence of wounds, haemorrhages, infections and parasites,*
- *behaviours like quick response to feed and fast swimming*

Cobia brooder

Pompano brooder

It is advantageous to collect sub-adults for broodstock development. Larger

fishes would have crossed the reproductive age and very small fishes will take longer time to sexually mature.

Collect cobia weighing between 8 to 15 kg for broodstock development. The pompano brooders could be procured in weight range of 750 gm to 1.5 kg.

Stress should always be minimised during capturing and handling of broodstock. It is best to collect broodstock fishes from trap nets, hook & line, etc., as they cause minimum stress to the fishes. Adequate dissolved Oxygen (DO) should be ensured during transportation.

Quarantine treatment

Upon arrival at the hatchery, broodstock fishes are released into the quarantine tanks for prophylactic treatment. Fish Anaesthetics like MS 222 (50-100 ppm) and Aqui-S (4 ml / 100 L), can be used for broodstock handling. The prophylactic treatment is given to limit the risk of introducing parasites or bacterial diseases into the hatchery facility. Short time exposure of brooders (maximum 5 minutes) in freshwater will help to remove the external parasites. The prophylactic treatment in hatcheries includes a sequence of medicated baths in formalin, malachite green and Oxytetracycline (OTC). Prophylactic treatment can be repeated three to four times within a week.

It is preferable to have a flow-through water circulation in quarantine tanks when treatments are not underway. Smooth inner surface in tanks allow easy and complete cleaning.

Following sequence of treatments can be followed:-

Day 1:

Fresh water bath for 10 minutes and then Oxytetracycline treatment (50 ppm) in seawater for 30 minutes.

Day 2 to Day 7:

Treatment with a mix of 200 ppm formalin and 0.2 ppm

malachite green for 1-2 minutes, followed by a freshwater dip for 5 minutes. Before returning the fishes to quarantine tanks with filtered seawater, they can be given an Oxytetracycline treatment at 50 ppm for 30 minutes.

The fishes should be closely observed during treatments. If the fishes suddenly become immobile or are found with very less opercular movements or are turning upside down, they should be immediately transferred to filtered seawater.

During the quarantine, fish should be closely monitored and fed during the day time when it is not undergoing treatment. Over feeding should be avoided and the fishes can be transferred to maturation tanks after the treatments are over.

Apart from quarantine treatment, the broodstock fishes should be given regular prophylactic treatment with freshwater with or without OTC at least once in a month.

Broodstock Development and maturation

After quarantine, broodstock fishes are moved into 100 tonne capacity RCC tanks for maturation and long-term holding in the hatchery. During gonadal maturation, water salinity needs to be 31-35 ppt. Water quality parameters like salinity, temperature, dissolved oxygen, pH, ammonia, and fish stock condition *viz.,* general behaviour, feeding activity, diseases symptoms, prophylactic treatments, etc. are monitored regularly. Normally sex ratio of 1 female: 2 males are maintained for cobia while it is 1:3 for pompano.

Broodstock development is the vital and time consuming procedure in marine finfish seed production. It is not easy to obtain broodstock fish directly from the wild and hence broodstock development is to be done in captivity. Pre-adults can be collected from the wild and maintained in sea cages or

Cobia broodstock fishes

Broodstock tank photos

recirculation facility. Broodstock development in sea cages was successfully done for cobia at Mandapam Regional centre of CMFRI. Circular cages of 6m diameter and 3.5 m depth with HDPE frame were employed for the purpose. The major risk in the development and maintenance of the broodstock in sea cages is the risk of contracting diseases and subsequent loss of broodstock. The sudden loss of broodstock will affect the seed production, since, the loss of broodstock cannot be made good from the wild immediately. Hence, on shore facilities like recirculation aquaculture systems is advised for development and maintenance of biosecured broodstock. The vital aspects which affect development of broodstock are the photoperiod, temperature and broodstock nutrition. In a shore based facility, the photo thermal conditioning can be practiced which will accelerate the gonadal maturation. In addition, it is also possible to obtain year round spawning in such a controlled system.

Recirculation Aquaculture System (RAS)

A Recirculataring Aquaculture System can be defined as an aquaculture system that incorporates the treatment and reuse of water with less than 10% of total water volume replaced per day. The concept of RAS in to reuse of water through conditional treatment and delivery to the organsims being cultured. The Virginia Tech, USA, has conducted industry-oriented research with cobia based on the application of recirculation based life

support systems. Further, researchers at the University of Texas at Austin Marine Science Institute's Fisheries and Mariculture Laboratory also spawned cobia in recirculating systems under photo-thermal controlled conditions. Besides a successful recirculating aquaculture system for maturation of cobia in captivity is also reported by researchers.

The Aquaculture Group of Virginia Tech (VTAG) initiated the development of an international initiative for sustainable and bio-secure aquafarming (IISBA) in 2005 and cobia was the first species that IISBA chose to address. The IISBA identified that the most significant and immediate bottleneck for cobia commercialization was fingerling production in required quantity.

The recirculation system proposed is with following minimum standards for design, construction and management.

1) Mechanical filtration: It is to remove the solids. It is usually designed a) to incorporate treatment streams for settleable (more than 100 micron) and suspended solids (less than 100 microns), b) to treat all recirculated water to a minimum of 100 micron and if possible 20 micron, and c) to incorporate facilities of cleaning and back washing with minimal water usage.
2) Biological Filtration: It is proposed to use biological filter of appropriate dimension. This system normally employs plastic biological filter media to provide adequate surface area for nitrifying bacteria. The water flow rates expected through the biological filters should exceed 200 % of recirculation system volume per hour.
3) Disease control: Disease control systems are proposed to be incorporated in all RAS as an essential water treatment element for management of fish health. Ultraviolet irradiation and ozone are used for disease control in RAS.

4) Oxygen management: The cobia fish require high dissolved oxygen (DO) content and hence DO below 5 mg/l will result in stress. Therefore, the required DO level should be maintained in the culture water by employing air blower / oxygen cylinders.

5) Carbon dioxide management: A degassing device is also proposed to be added for management of carbon dioxide.

6) Culture tanks and pumps: The RAS incorporates suitable RCC / FRP culture tanks. The RAS incorporates industrial pumps constructed of corrosion resistant materials.

7) Foam fractionation: It is required to incorporate a foam fractionation system to remove fine solids and dissolved organic materials.

8) Thermal control: The RAS should have thermal control mechanisms to maintain appropriate temperature for broodstock maturation and spawning.

9) Supporting infrastructure: Should have supporting infrastructure for photo-period control to facilitate controlled maturation of cobia / pompano.

Broodstock Nutrition

The viability of the larvae is very much dependent on broodstock nutrition. The nutritional components in the diet, the feed intake rate or the feeding period can all affect spawning, egg and larval quality. In the case of tropical fishes, ovarian development is often asynchronous – oocytes in all stages of development are present at the same time and sometimes independent of season. The ovarian development starts with the formation of primary oocytes. During the primary growth phase, the surrounding granulosa and theca cells envelop the oocyte to form the ovarian follicle. In the early stages of secondary growth, cortical alveoli appear and accumulate in the periphery of the

oocyte. Even though the oocyte may increase in size several fold during primary and early secondary growth, the most conspicuous size increase occurs during the last part of secondary growth, vitellogenesis. Vitellogenesis is the process of yolk formation and incorporation in the growing oocytes. The yolk protein precursors, vitellogenins, are high molecular weight lipoproteins that are synthesized in the liver and secreted into the blood. The fatty acid composition of the vitellogenins can be affected by long term imbalances in the broodstock diet. It has been well established that feeding broodstock fish with squid, cuttlefish or meals made from cephalopods have beneficial effects. These feed ingredients make the diet more attractive and therefore increase feed intake. Squid and cuttlefish also contain high levels of essential fatty acids.

For quicker maturation, the broodstock fishes are to be fed with highly nutritive diet. Diet rich in vitamins, poly-unsaturated fatty acids (n- 3 PUFA) and other micro-nutrients is essential for obtaining viable eggs and larvae. During gametogenesis, female fish require a food, richer than usual, in proteins and lipids to produce the vitellogenin. As the sole source of food for the developing embryo and the early larval stage until feeding on live preys starts, yolk quality and quantity are key factors for a successful reproduction. Both dry pellets and moist food are also employed during maturation. Dry pellets should include essential nutritional components like polyunsaturated fatty acids (n-3 PUFA), in particular EPA (20:5 ω 3) and DHA (20:6 ω 3),

Artificial Broodstock feed

Trash fish

Squid

which cannot be produced by fish metabolism . Broodstock fishes are fed *ad libitum* once a day with squids, cuttlefish, crabs, shrimps and chopped oil-sardines depending on the availability.

Tagging of Fish

Tagging or physical marking of broodstock fishes through easily detectable methods is very much essential for selection of broodstock for identification, selective breeding and segregation. The most popular method is PIT Tagging.

What is PIT tag?

Passive Integrated Transponder (PIT) tag, also known as is a radio frequency device to permanently mark fishes internally. The tag is designed to last throughout the life of the fishes providing a reliable, long term identification method.

Principle of PIT tag

The PIT tag contains a microprocessor chip and antenna. It has no internal battery, hence the term "passive", so the microchip remains inactive until read with a reader. The reader sends a low frequency signal to the microchip of the tag providing the power needed to send its unique code back to the reader and therefore fish is positively identified.

Read range and frequency of the tag

The distance from which a tag can be read is the read range. Most read ranges using hand-held readers are 3 to 9 inches depending on the reader. There are currently three basic tag frequencies. The 400-kHz tag was one of the first developed but it has limited read range. As microchip technology evolved, the 125-kHz and 134.2-kHz tags became available. Compared to the older 400-kHz tags, they have a much better read range and reduced read time. The 134.2-kHz tag was developed to meet international standards for code format. It is very much

important that the tag type and reader unit should be compatible. Most readers are capable of detecting both 125-kHz and 134.2-kHz frequencies.

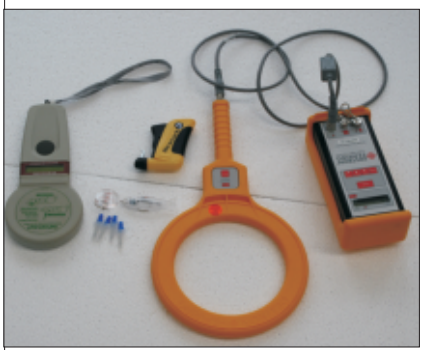
Tags can be read through materials such as soil, wood and water. Ferrous metals and noisy environments can cause interference between the electromagnetic communication of the reader and the tag.

Durability of the tag

Design engineers' calculations suggest that PIT tags can last as long as 75 years or more. There is no battery to fail and the glass encapsulation is impervious to almost everything. PIT tags can be removed or recovered from a primary location and reused indefinitely.

Safety to animal

Reducing stress to the fish is the prime factor in ensuring the success of the tagging and safety of the fish. Therefore, the fish should be anesthetized during the implantation of PIT tags. Species, size and age should be considered when making a decision about anesthetization and restraint. Sterile implants are advised but many field conditions do not allow for sterile implants. Equipment can be disinfected prior to use with alcohol



PIT tagging equipment

Tagging of cobia

and iodine-based solutions. The tag is encased in glass that protects the electronic components and prevents tissue irritation, thereby very much safe to the fish.

Advantages of PIT tag over other tags

- Highly reliable individual identification
- Permanent identification marker
- Small size and no interference with the behaviour of fish
- No error in recording data
- Rapid data collection

Disadvantages

- Initial cost is high
- Low detection distance

PROCEDURE OF TAGGING

Site of implantation

The implant site depends upon the species, size of the fish and the size of the tag. It is preferable to implant the tag on the dorsal musculature of the fish which will be convenient for the brood fishes to be read.

Stepwise protocol

- Use sterile needle or implanter to tag the fish. In field condition, disinfect all the components prior to use with alcohol and iodine-based solutions.
- Read the tag before inserting into the fish and record the identification code or number.
- Catch the fish and anaesthetize it with suitable anaesthetic. In sea cages, it is easier to restrain the fish inside the catching net.
- Disinfect the site of implantation with alcohol or iodine-based solution.

- It is a better practice to keep a standard site of implantation so that the reading will be easier and quicker.
- The tag loaded inside the implanter has to be inserted into the muscle tissues. It is advisable to insert the tag parallel to the muscle fibres to avoid much damage to the tissues.
- The tag should be released slowly and steadily from the implanter while removing the implanter from the tissue in such a way that the tag fills the space created by the implanter needle.
- Once implanter needle is taken out, the site should be disinfected again with alcohol or iodine-based solutions to avoid secondary infection.
- Release the fish as soon as the tagging is over or once it has recovered from anaesthesia.

Maturation and spawning

The natural process of sexual maturation of the broodstock fishes can be accelerated by altering the photo-thermal period and it is also possible to obtain viable larvae almost throughout the year. At the onset of the spawning season, it is necessary to move selected broodstock fishes from maturation tank to spawning tank after assessing the ovarian development through cannulation. Only females with oocytes in the late-vitellogenic stage, with a diameter around 700 μm in cobia and 500 μm in pompano, are selected.

Ovarian biopsy can be carried out as follows :

- Female brooders have to be transferred to a small tank containing anaesthesia in sufficient quantity.
- Flexible sterile catheters (1.2 mm internal diameter) can be used for cannulation biopsy.
- Introduce the sterile catheter into the oviduct, up to the ovary for a few cm; then suck carefully a small sample of oocytes up

into the catheter and place the sample on a glass slide.

- After sampling, release the animal into the spawning tank, where recovery from sedation will take place.
- Put few drops of filtered sea water on the biopsy sample and examine under the microscope and measure the diameter of the oocytes and record the measurements.

Cannulation of cobia brooder

Induced spawning

Spawning can be obtained either naturaly or by inducing with hormones. Induced breeding is commonly practiced in most commercial hatcheries. The hormonal treatment is intended to trigger the last phases in egg maturation, i.e. a strong egg hydratation followed by their release. However, if eggs have not reached the late-vitellogenic (or post-vitellogenic) stage, the treatment does not work; hence ovarian biopsy is essential for assessing the ovarian development. The human chorionic gonadotropin (hCG) is used at a dosage of 500 IU per kg of body weight in cobia females and 250 IU per kg body weight for males, whereas, for pompano 350 IU per kg body weight is used for both male and female. This dosage can be administered as a single dose on the dorsal muscles. Use of hCG treatment sometimes gives serious setbacks like not all females respond to it, egg quality may

be below acceptable standards with hatching rate lower than 80%, being a large molecule it may provoke immunization reaction , and as a result, fish treated with hCG may not respond when treated repeatedly with this hormone. However, hCG can be successfully replaced by an analogue of the luteinizing hormone-releasing hormone [LH-RHa des-Gly10 (D-Ala6) LH-RH ethylamide, acetate salt]. It is a small molecule with 10 peptides and acts on the pituitary gland to induce the release of gonadotropins which, in turn, act on the gonads. Almost 100% of injected fish spawn eggs whose quality usually matches that of natural spawning.

The cost of LHRHa is very high compared to that of hCG. But, LHRHa is used in very low dosages, usually around 20 µg / kg of body weight

Hormonal administration to cobia

Spawning tanks

The spawning unit should preferably be kept separated from the main hatchery building to avoid disturbance to the spawners and possible risk of disease contamination. However, for economic reasons, it is usual to keep the brooders inside the hatchery in a specific dedicated area. Though we use only rectangular tanks based on availability, it is preferable to use circular tanks with at least 1.20 m depth. Shape and depth of tanks

count for easy and free movement of brooders. Normally the spawning could be noted within 36 -48 hours after hormonal induction. The spawning in cobia and pompano takes place normally between late night and early morning hours. The number of eggs spawned by cobia ranges from 0.4 to 4 million, whereas, the pompano brooders spawn 0.5 to 1.5 lakh eggs.

Egg harvest

The fertilized eggs of cobia and pompano float and are scooped gently using 500 μm net. To minimise the presence of poor-quality eggs, which usually float deeper in the water, it is advisable to collect only the eggs which float at the water surface. Therefore, aeration can be switched off allowing the unfertilized / dead eggs to settle at the bottom of the tank. The floating layer of eggs thicker than one cm should be avoided. A thicker layer may reduce oxygen supply to the eggs, leading to possible anoxia after a short time. Then in the temporary container, eggs must be thoroughly examined to assess their quality, number and developmental stages. With a pipette eggs should be taken from the floating egg layer in the temporary container, and should be placed on a watch-glass or on a Petri dish for observation under microscope. Few dozens of eggs, which are placed under a microscope or a transmitted-light stereomicroscope have to be observed for the egg developmental stages.

As fertilised cobia/ pompano eggs float in the seawater, they can be collected using egg collectors. If well dimensioned and properly placed, these devices harvest only the floating eggs, while the dead or unfertilised ones sink to the bottom. The presence of eggs in the collectors should be checked rather frequently in the case of cobia, as its spawning releases a large amount of eggs in a very short time there is risk of clogging the collectors leading to mechanical stress to the eggs.

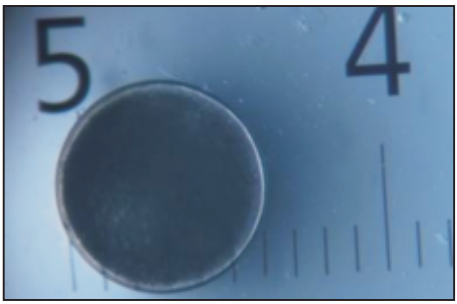
Check for the following egg characteristics:

- presence of opaque, whitish eggs which are unfertilised. Similarly, eggs in the sample with transparent, but without evidence of cell divisions
- regular rounded shape and size (diameter 900-1000 mm in cobia; 800 -900 mm in pompano), regular cell division that can be observed only in the first blastomers; regular shape of yolk (it should occupy the egg volume entirely, without perivitelline space),
- absence of parasites or associated micro-organisms on the chorion surface.

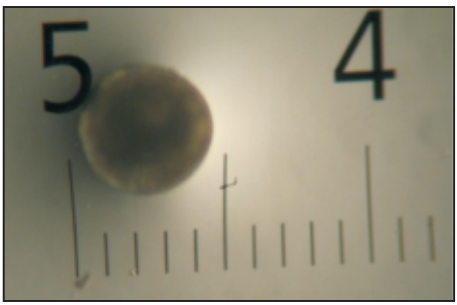
Incubation of eggs

Incubation of eggs is done in tanks of 3-5 tonne capacity. After hatching, the larvae have to be moved to the larval rearing tanks filled with filtered seawater. Prior to this, the aeration should be stopped briefly to enable the debris and exuviae to settle at the bottom which can be removed by siphoning. Aeration needs to be adjusted suitably, not too strong to avoid excessive physical collision among eggs, but not too weak either, to keep the eggs suspended in water column. The main purpose of aeration is to prevent clumping and settling down of eggs. Air bubbles should not be too small as seen while using air diffusers instead of stones, as it results in clumped eggs and damage of the eggs. It is suggested to limit as much the number of air stones as possible. Stocking density can be maintained at a moderate level of 200 to 500 eggs per litre. The development of embryo can be observed at frequent intervals under a stereo / compound bionocular microscope. The hatching of eggs takes place from 18 to 24 hours.

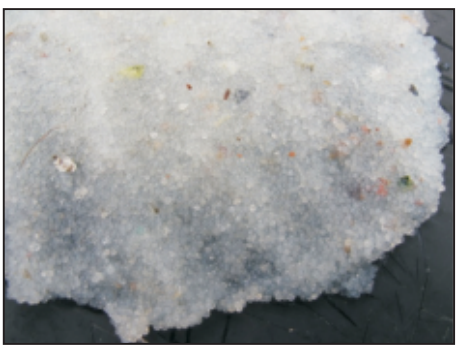
As the fecundity is normally high in cobia, we may require more incubation tanks, whereas the pompano requires only one tank / female.



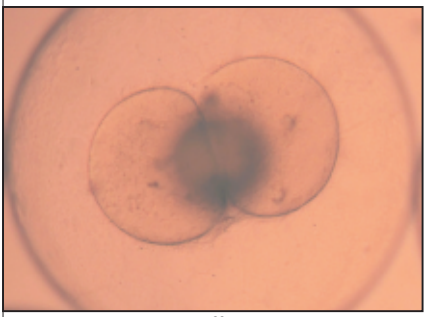
Good quality egg



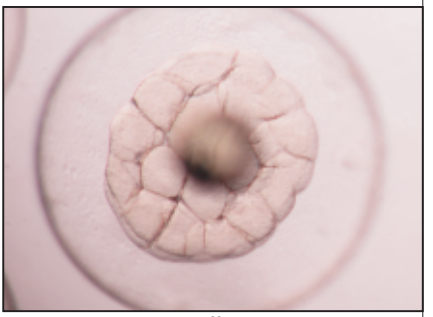
Under developed eggs



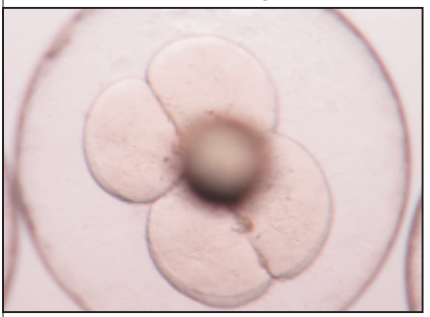
Unfertilized eggs



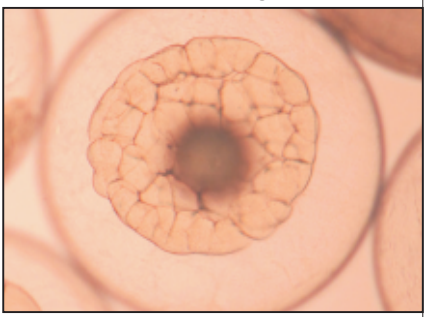
Two-cell stage



32 - cell stage

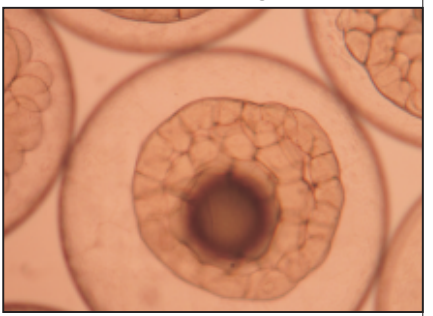


Four - cell stage

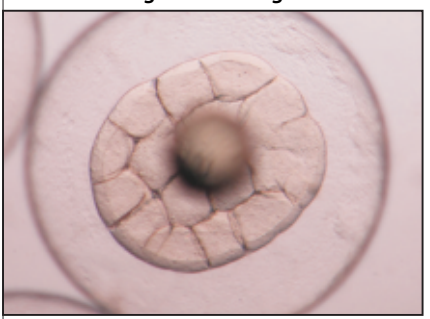


64 - cell stage

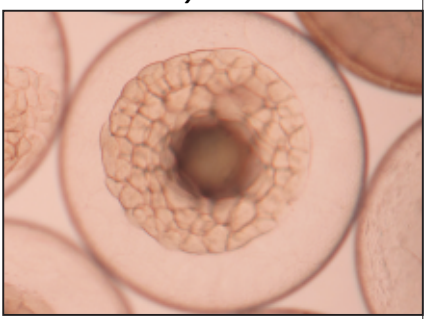
Eight - cell stage



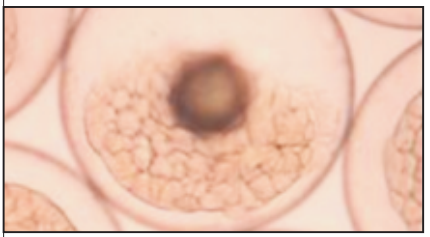
Early Morula



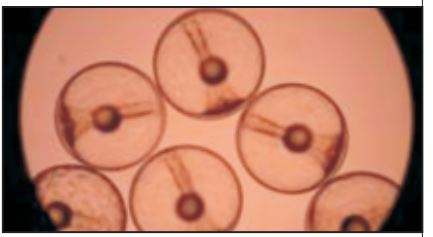
16 - cell stage



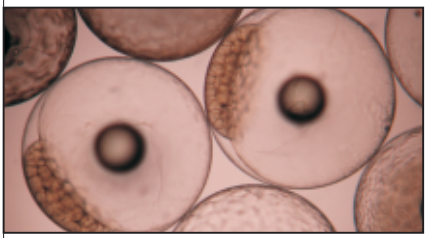
Late Morula



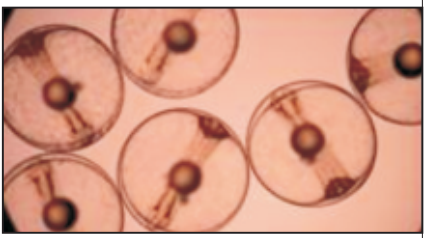
Early Blastula



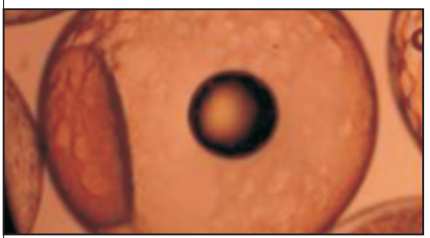
Early Gastrula



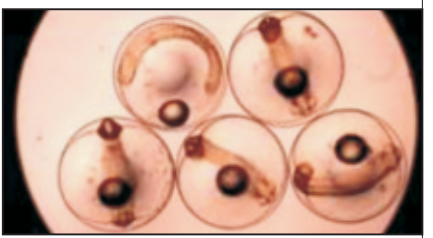
High



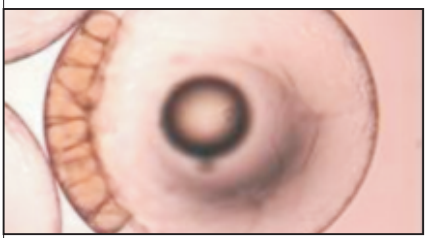
Mid Gastrula



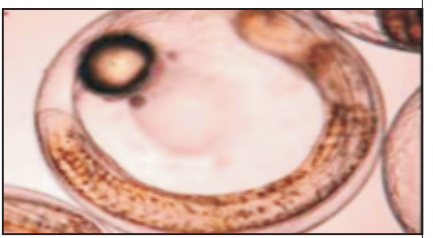
Dome



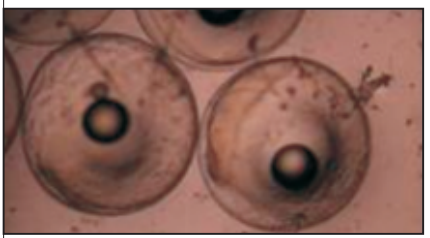
Late Gastrula



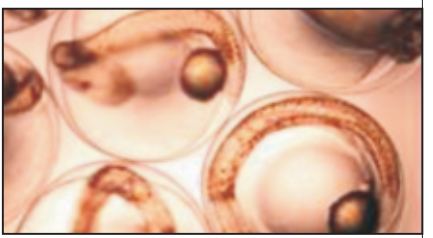
Oblong



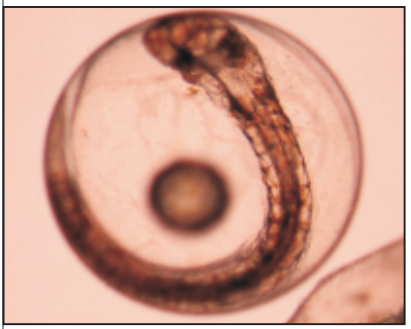
Bud



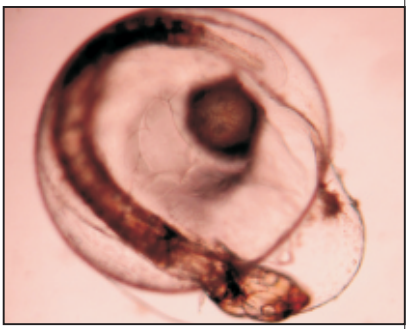
Epiboly 30%



Segmentation

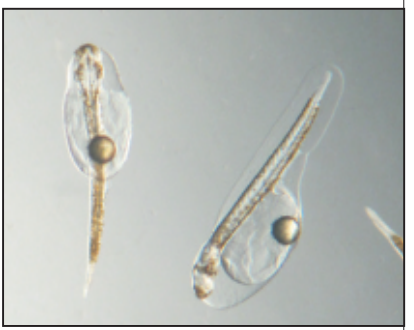


High-pec

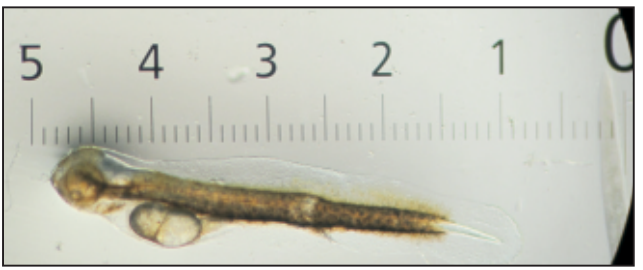


Hatching in progress

Newly hatched larva



Larvae-12 hour post hatch



2dph

Larviculture

The marine fish larvae are generally classified into altricial and precocial type. The altricial type of larvae are having very less yolk reserves at hatching and hence, the larvae are in a undeveloped stage when the yolk sac is completely resorbed. The development of digestive system is also very primitive in these types of larvae. Many of the marinefish species suitable for aquaculture are characterized by having the altricial type of larvae which pose challenges in their larviculture. When the yolk reserves are fully exhorted, the larval size and mouth gape are very small and the perceptive powers for searching and taking external feed is also very less. The period when the yolk reserves are fully exhausted and larvae need to resort to exogenous feeding is a critical period in the larviculture of most marine fin fishes. Unless proper live feeds of required size is provided in sufficient densities in larviculture media and its nutritional requirements especially in terms of PUFA are met, large scale mortality is bound to happen at this stage and hence it is evident that the larviculture of marine finfish having altricial larvae is really challenging and proper management of live feed is the most vital pre-requisite for the success in terms of survival and growth of the larvae.

In addition, since most of the larvae are visual feeders providing the required light also affect the larval survival. During the critical period, the density of the live feed and its nutritional qualities determines the percentage of the survival of the larvae. The density of the larvae of the concerned species should also be regulated in the larviculture tanks for getting good survival. The marine fish larvae exhibit highly differential growth even from very early stages (in the case of cobia, starting from the first week) and hence grading from an early stage is also very much needed for increasing the survival. In addition, variety of other factors such as tank colour, size of the tank, water temperature, water quality, etc., affect the larval survival and growth. From the foregoing, it is clear that the larviculture of marine finfish is

highly complicated, unless each and every factor is taken care of, the survival and growth of the larvae will be very meager.

Newly hatched larvae have to be checked to assess their viability and condition prior to stocking in the larviculture tanks. At least 10 to 20 fish larvae have to be observed under the microscope for the following:-

- shape and dimensions
- deformities, erosions and abnormalities
- appearance of internal organs
- pigmentation
- absence of external parasites

The larvae hatched in the incubation tanks or larval rearing tanks need to be distributed in larviculture tanks to have minimal stocking density of 5 to 10 larvae/ litre for cobia and 10-20 larvae per litre for pompano. Care should be taken to avoid any mechanical stress or damage. Soon after hatching, the mouth is closed and the digestive tract is not fully developed. During this period the larvae survive on its reserves in the yolk sac.

Larviculture of cobia

Newly hatched larvae of cobia normally measures 3.4 mm size. Larval mouth opens at 3-5 days post hatch (dph). Metamorphosis starts from 9-11 dph. Newly hatched cobia larvae generally start feeding at 3 dph and they can be fed with the enriched rotifer (*Brachionus rotundiformis*) at the rate of 10-12 nos / ml, two times a day till 10 dph. From 8 dph, the larvae can be fed with enriched *Artemia* nauplii at the rate of 2-3 nos / ml, 2 times a day. During the rotifer and *Artemia* feeding stage, green water technique can be used in the larviculture system with the microalgae *Nannocloropsis occulata* at the cell density of $1x10^7$ cells / ml. The weaning to artificial larval diets has to be started from 15- 18 dph. While weaning, formulated feed should be given 30 minutes prior to feeding with live feed. Size of the artificial feed has to be smaller than the mouth size of the fish. Continuous water

exchange is required during weaning stage. Between 25-40 dph, the larvae are highly cannibalistic and hence size-grading has to be undertaken at every three days interval. During this stage, the fry could be weaned totally to artificial diets. Larval rearing can be practised both intensively in tanks and extensively in ponds. The major factors affecting the growth and survival of larvae are nutrition, environmental conditions and handling stress. Since there is high demand for essential fatty acids (EFAs), enrichment protocols are needed for live-feeds. The water exchange can be practically nil till 7dph and it can be gradually increased from 10-100 % from 8 to 12 dph. But, tank bottom siphoning should be carried out from day 1. The environmental conditions required during the larviculture period are DO_2: > 5mg / L , NH_3: < 0.1mg / L, pH: 7.8 – 8.4, Salinity: 25-35 ppt, water temperature : 27-33° C.

Green water has to be maintained in appropriate densities in the larval tanks. While weaning the fish larvae from rotifers to *Artemia* nauplii, co-feeding with rotifers has to be continued due to the presence of different size groups of larvae. The detail of weaning protocol is as follows.

Age of Larvae (dph)	Size of Larvae (cm)	Size of Feed (µ)
18 – 19	2.3 – 2.6	100-200
20 – 23	2.5 – 3.5	300-500
23 – 30	3.5 – 8.0	500-800
31 onwards	> 8.0	800-1200

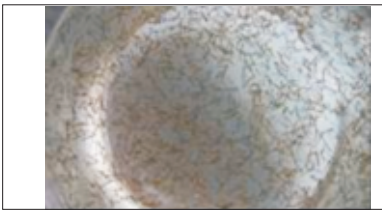
The juveniles measuring 10 cm length were ready for stocking in happas/ nursery tanks.

Nursery and grow-out rearing of cobia

Nursery phase of cobia can be carried out in happas or sea cages or indoor FRP / cement tanks. During nursery rearing, it is advisable to feed the juveniles with formulated feed of 1200 µ size which can be increased to 1800 µ size from 55 dph onwards. Once the juveniles reach a size of 15 gm, they are ready to stock in sea cages or land based ponds for grow-out farming.

Larval stages of cobia

3 dph



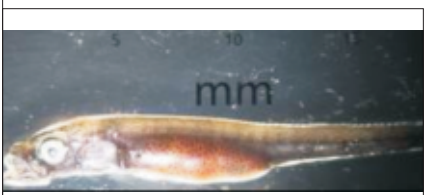
4 dph

6 dph

10 dph

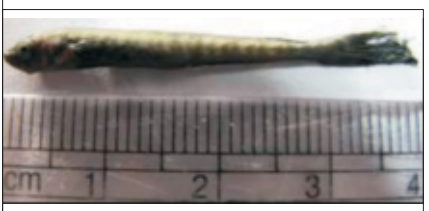
13 dph

15 dph



20 dph

21 dph



25 dph

30 dph

Larviculture of Pompano

The newly hatched larvae were stocked at a density of 15000 larvae in FRP tanks of 2 m^3 capacity filled with 1.5 m^3 filtered seawater. The tanks were provided with mild aeration and green water at a cell density of 1 x10^7/ml. The mouth of the larvae opens on 3dph and the mouth size was around 230 µ.

The larvae were fed from 3dph to 14 dph with enriched rotifers at a density of 6-8 nos. per ml in the larviculture tanks. Wherever possible, wild collected copepods could also be added as supplements. Co-feeding of rotifers with enriched *Artemia* nauplii has to be done during 12-14 dph, and thereafter upto 19 dph with enriched *Artemia* nauplii alone by maintaining a density of 3-5 nos. per ml in the larviculture tanks. Weaning to larval inert feeds has to be started from 15 dph and co-feeding with *Artemia* needs to be continued until 19 dph . From 20 dph feeding can be entirely on larval inert feeds. The metamorphosis of the larvae starts from 18 dph and all the larvae metamorphose into juveniles by 25 dph. Though cannibalism is not witnessed, grading has to be done during 20-25 dph to separate the shooters. Critical stage of mortality would occur during 3-5 dph and subsequent mortalities are negligible. The water exchange can be practically nil till 7dph and it can be gradually increased from 10-100 % from 8 to 14 dph.

Nursery Rearing of Pomapano

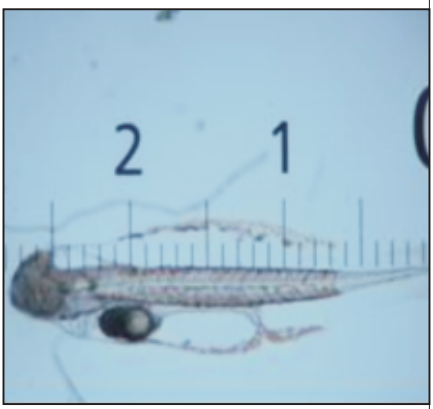
Nursery rearing could be initiated from 25 to 30 dph. At this stage, artificial feed of 800µ size could be provided. Thereafter, fingerlings were fed with progressively higher size range of floating extruded larval feeds. Daily water exchange of 100% is advisable. Water quality parameters like salinity, temperature, pH, Oxygen level and ammonia are closely monitored during the entire larviculture period. After 55dph, the fingerlings with size range from 1 to 1.5 inch size can be supplied to farmers for stocking in the

happas / tanks for further nursery rearing and grow-out farming thereafter.

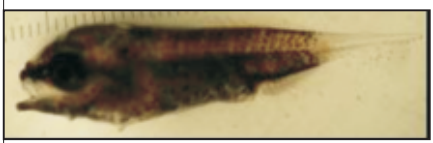
The pompano fingerlings can be reared at salinities as low as 5 ppt. At lower salinities from 10 to 15 ppt, they grow faster than in pure seawater.

Larval stages of pompono

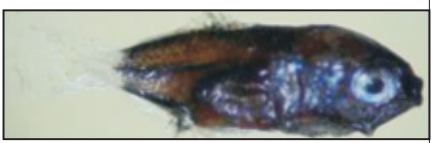
Newly hatched larva



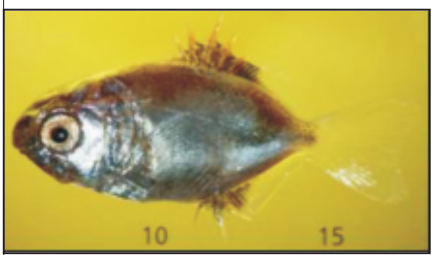
Larva on 2 dph



3 dph



13 dph



18 dph

28 dph

Application of larval feeding behavior in the larval rearing technology

After egg hatching, fish larvae go through important changes to reach the juvenile stage, the most evident being a dramatic biomass increase. Feeding success in fish larvae is critical for obtaining the nutrients and the energy necessary for healthy growth and development that allows them to survive to the end of the larval period.

Feeding behavior is the result of interaction of complex processes *viz.* searching, detection, attack, capture, ingestion, digestion and excretion. Each of them has a specific pattern that changes throughout development. The feeding strategy is related to the specific characteristics of each species. Availability of suitable prey is one of the most determinant biotic factors, but feeding mode and amount of food intake are also influenced by prevailing environmental conditions.

Searching and detecting food

Searching for prey and detecting them depend on the appropriate functioning of some organs and tissues that become progressively available throughout development. From hatching, larvae are progressively aware of different external stimuli that indicate the presence of potential food items. Searching depends basically on swimming capacity, while detection depends largely on sensory organs. Food detection occurs by means of visual, chemical and mechanical stimuli. Olfaction allows detection of distant stimuli, sight allows the identification of objects at medium and relatively short distance , while touch needs very close or direct contact with the source of stimulus. Most marine fish hatch with immature anatomical features. The sensory organs develop quickly during the first days after hatching. Sight allows the larvae to perceive objects that are relatively close. Altricial marine teleosts hatch with undeveloped eyes although the pigment in the retina

appears in a few hours or days. This early retina has only one type of photoreceptor that allows vision only under bright light. Double and mosaic cone structures and rod photoreceptors appear later and enable vision at low light intensity. Olfaction allows for more remote detection of a stimulus. The olfactory organ appears early during embryonic development. Olfactory placodes and pits are already present at the onset of feeding and develop further by the late larval stage. The intra and extra oral taste buds develop or proliferate some days or weeks after the first feeding. Mechanical stimuli such as touching or water movements are detected by neuromasts and the lateral line system. In larval fish some few free neuromasts are already present at hatching and progressively proliferate during their growth and development. The progressive development and completion of all these sensory organs increase the capacity for detection and recognition of potential prey.

Locomotor capacity

Basically fish larvae exhibit alternating periods of swimming ability and inactivity. Swimming speed, pause duration, reactive distance, perception angles and duration of predation cycle define the changes in behavior during searching and attack throughout development. At first feeding, even the smallest larvae have some primordial hunting habits , but the efficacy increases with development and growth, changing from passive feeding to an active prey searching capacity.

Capture and ingestion

Capture success relies not only on development stage and concomitant hunting capacity but also on the availability and accessibility of prey. Once the prey is perceived, the foraging has three possible results: unsuccessful attacks, aborted attacks and successful attacks. After mouth opening, fish larvae need to learn hunting and have to do it quickly. High prey availability and accessibility are crucial for successfully initiating feeding. Prey size

and swimming ability are primary factors determining the efficacy with which the prey is caught. The ability to start feeding after mouth opening is typically affected by prey size. During the very early stages with low swimming capacity, encounter opportunity depends on prey density. Mouth gape limits the dimensions of the prey that can be ingested. Prey/gape ratio determined in different species usually ranges between 25 and 60%. Searching for appropriate prey of adequate size has been a priority for rearing fish larvae. The established prey sequencing is based on rotifers of different sizes and Artemia nauplii and meta nauplii. However, there is a need to search for live feeds below 100 micron size for rearing of very small marine fish larvae. Eventhough copepod nauplii can be employed for this purpose, mass scale production of copepod nauplii for large scale larval rearing is a major constraint. Overall , the current commonly used live feeds, *Brachionus* spp. and *Artemia* spp. meet well the feeding behavior of larvae except very small larvae at mouth opening.

Live feed culture

Live feeds are the main items in the diet of cultured fish larvae and they are of particular importance when rearing marine fish larvae of the altricial type. Altricial larvae are those that remain in a relatively undeveloped state until the yolk sac is exhausted. At first feeding the digestive system is still rudimentary, lacking a stomach, and much of the protein digestion takes place in the hind gut epithelial cells. Such a digestive system is in most cases incapable of processing formulated diets in a manner that allows survival and growth of the larvae comparable to those of live feeds. Despite the recent progress in the development of inert diets for fish larvae, feeding of most species of interest for aquaculture still relies on live feeds during the early life stages.

The low digestive capacity of the altricial larvae might not be the only aspect responsible for them requiring live feeds. Live preys

are able to swim in the water column and are thus constantly available to the larvae. Most formulated diets tend to aggregate on the water surface or sink within a few minutes to the bottom and are thus normally less available to the larvae than live feeds. In addition, since larvae are visual feeders adapted to attack moving prey in nature, the movement of live feed in the water is likely to stimulate larval feeding responses. Live prey with a thin exoskeleton and high water content may be more palatable to the larvae once taken into mouth, compared with the hard, dry formulated diet.

Microalgae

Microalgae constitute the first link in the oceanic food chain. In aquaculture, microalgae are produced as a direct food source for various filter feeding larval stages of organisms. They are also used as an indirect food source in the production of rotifers, Artemia and copepods which in turn are used as food for the carnivorous larvae of many of the marine fish species. For rearing marine fish larvae according to the 'green water technique' microalgae are used directly in the larval tanks. This technique is nowadays a normal procedure in marine larviculture and is reported to improve fish larval growth, survival and feed ingestion. The role of microalgae in the rearing water is attributed to (i) providing of nutrients directly to the larvae (ii) contributing to the preservation of live prey nutritional quality (iii) promoting changes in the visual contrast of the medium and its chemical composition and (iv) playing an important role in the microflora diversification of larval gut.

Whenever microalgae are used as a direct food source or as an indirect food source in the production of rotifers, Artemia or copepods, growth of the animals is usually superior when a mixture of several microalgal species is used. This probably occurs as different species compensate one another for eventual deficiencies in given nutrients. Special care is needed when selecting microalgae for on growing live feeds for marine fish larvae, in order to avoid the

nutritional deficiencies of the latter especially in terms of n-3 highly unsaturated fatty acids. Deficiencies in the n-3 PUFA contents of microalgae may cause severe mortalities and quality problems in marine fish larvae. Such deficiencies may also cause reduced fecundity of rotifer and copepod cultures.

Recently commercial microalgal products are developed which can also be effectively employed for larviculture. These include microalgae concentrates, frozen and freeze dried microalgae and microalgal pastes. Results of these products are generally good.

Microalgae like *Chlorella sp., Nannochloropsis sp., Tetraselmis sp., Dunaliella sp*., and *Pavolova sp*., *Isochrysis sp.* can be used as algal diet for growing the rotifers. The size, nutritive value, proliferation rate and digestibility of the algae are the critical factors for selecting the algae for the use in marine hatchery use.

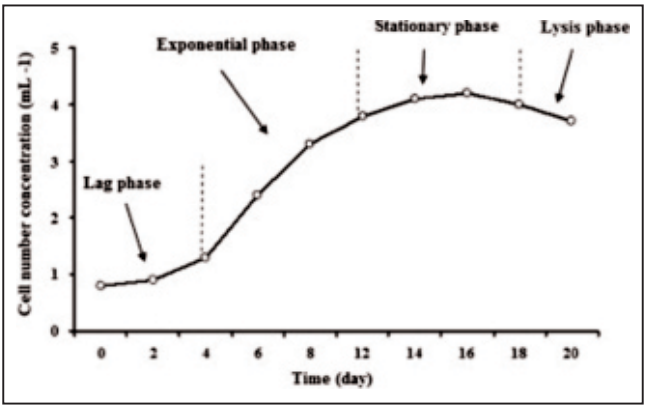
Nutrition required by Microalgae

- Macro elements include nitrate and phosphate as prominent nutrients
- Micro elements are iron, molybdenum, copper, zinc, cobalt, B1 (thiamine), B12 (cyanocobalamin) and biotin.

Growth phase of Microalgae

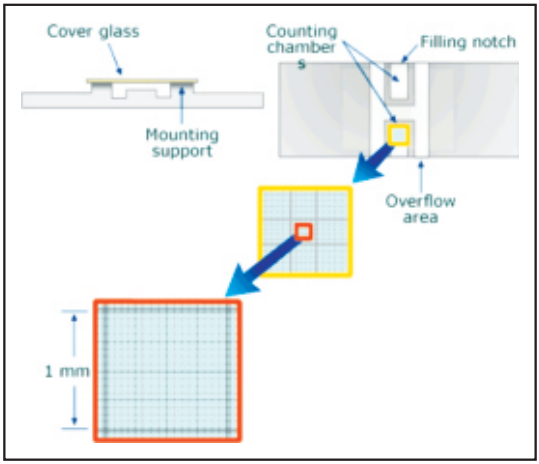
Microalgae grow by normal cell division and cultures supplied with excess resources will normally exhibit an exponential increase in cell numbers. After inoculation, the cultures need to acclimate to the new culture condition and the microalgae culture will have a lag or induction phase. When the cells have become acclimatised to the conditions of excess resources, they grow and divide faster and the culture will follow an exponential growth phase, the cell density increases, while the nutrient in the growth medium will be exhausted and the increased density of the algal

cells will increase self spreading of the culture. The culture will then come into a phase of declining growth rate before it reaches the stationary phase. In the stationary phase, the net increase in cell numbers is zero. After some time depending on the species, the culture will continue into the death phase, where mortality rate exceeds growth rate.



Measurement of Algal Density

The algal cell density is measured using haemocytometer. The haemocytometer is a specialized microscope slide on which 2 grids have been engraved, in a central region that is 0.1 mm lower than the rest of the slide. Each grid comprises 25 large squares, each containing 16 smaller squares of area 1/400 mm^2. This creates a region of known volume (0.1 mm^3) when a special coverslip is correctly placed over the central region (get someone to show you how to do this - and take care as the cover slips are easily broken). 10µl of culture are then pipetted under the coverslip and cells counted in a proportion of the grid squares (count as many as is convenient). Multiplying the total number of cells in the entire grid by 10^4 gives the number of cells per ml.



Algal Culture Management

STAGE I - (Agar culture, test tube and Erlenmeyer 100 - 250 ml, 500 - 1000 ml)

- The turbidity of media is approximately zero/nil which can be achieved by filtering water with 5 µm and 1 µm cartridge filters and UV filter.
- Salinity can be decreased (28-30 ppt) with the addition of 10 % distilled water (V1N1 = V2N2).
- Use only laboratory grade media.
- Autoclaving is mandatory
- Incubation has to be done at 19^{0}C-23 ^{0}C and 1 or 2 neon lamp with 40 watt each has to be provided above the culture tank.
- One or two drops starter algal culture in a ratio 1 to 5 or 1 to 10 has to be added.

STAGE II (bottle culture 1000 ml)

- The turbidity of media is approximately zero/nil which can be achieved by filtering water with 5 µm and 1 µm cartridge filters and UV filter.

- Salinity has to be maintained at 30 - 32 ppt
- Laboratory grade fertilizer can be used in a ratio of 1:0.5:0.1
- Sterilization with chlorine 10 ppm has to be done and it can be neutralized with $\leq$ 5 ppm of sodium thiosulfate.
- Incubation at 24 °C with two neon lamps with 40 watt each is required.
- Starter algae can be added in a ratio 1 to 2 or 1 to 5
- Incubation of 21 to 25 days is required.
- Frequent mixing of algal culture by thorough shaking of the flask is required.

Laboratory Scale media (for stock culture upto 2000 ml flask)

STAGE III (Carboy culture 20 liter)

- Salinity of media can be 31-32 ppt

Walne Media		
$NaNO_3$	: 100 g	A-solution
Na_2EDTA	: 45 g	
$Na_2H_2PO_4 2H_2O$	: 20 g	
$FeCl_3 6H_2O$	: 1.3 g	
$MnCl_2$	: 0.36 g	
H_3BO_3	: 33.6 g	
Vitamin B1	: 0.1 g	B-solution
Vitamin B12	: 0.005 g	
$ZnCl_2$	: 2.1 g	C-solution Ratio 1:0.5:0.1
$CoCl_{12} 6H_2O$	: 2.0 g	
$(NH_4)Mo_7O_{24} 4H_2O$	: 0.9 g	
$CuSO_4 5H_2O$	: 2 g	

- Use laboratory grade fertilizer (ration 1:0.5:1)
- Sterilization with chlorine 10 ppm and neutralization with sodium thiosulfate (5 ppm)
- Temperature has to be maintained at 24 °C and lighting with two neon lamps (40 watt each)
- Add algae starter with ratio 1 to 7
- Supply of CO_2 using aeration
- Incubate for 5 to 7 days

STAGE IV (Intermediate Culture in an aquarium with volume of 100 liter)

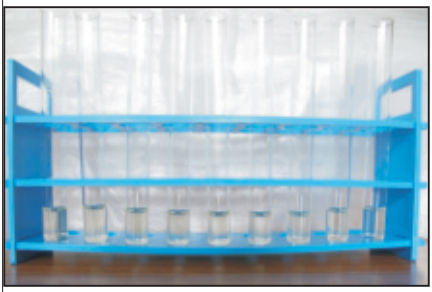
In outdoor

- Salinity 32-33 ppt
- Use commercial grade fertilizer
- Chlorine sterilization 5 -10 ppm
- Use filter 1 micron Filter bag to filter the culture water
- Vigorous aeration is required
- Temperature 29-30^0 C
- Expose in direct sun light
- Incubate for 5 to 7 days

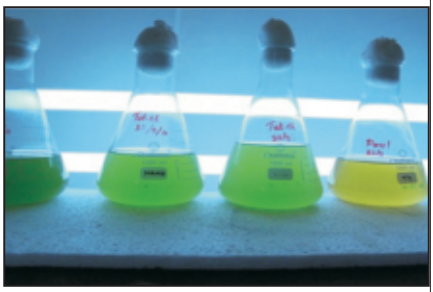
STAGE V (Intermediate fiber glass culture in 1 m^3 volume)

In outdoor

- Salinity media 32 - 33 ppt
- Use commercial grade fertilizer
- Sterilized with chlorine with dosage of 10 ppm
- Use filter 1 micron Filter bag to filter the culture water
- Vigorous aeration is required
- Expose in direct sun light
- Incubate for 5 to 7 days

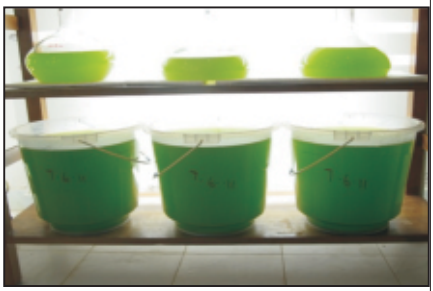


Algal culture in test tubes



Algal culture in conical flasks

Algal culture in flasks



Algal culture in carboys

Outdoor culture

MASS CULTURE OF ALGAE

Production Systems

Common micro algal production strategies use either batch cultures or continuous cultures. A batch culture follows a typical growth curve, starting with an inoculation phase, an exponential growth phase and further into a stationary phase, where the carrying capacity of the culture is reached. Batch cultures are started at a low density and are harvested when desired density is achieved.

Continuous culture means that the cultures are harvested more or less continuously. There are several types of continuous cultures- the chemostat and semi continuous culture. In both a specific volume is harvested each day and the harvested volume is replaced by new growth media. A chemostat culture is continuously harvested and will have stable conditions of light, nutritional state etc., over time and the produced iomass and composition of algae can be practiced. Recently, hatcheries with requirements for microalgal produce it by photo bio reactors built up by horizontal or vertical tubes. The benefit of such reactors is that they have very large surface in relation to the volume, with good supply of light energy to the culture.

- The objective of mass plankton culture is to fulfil the necessity of zooplankton feed (rotifer) and to supply into the larva tank as the enrichment of zooplankton feed, to support the water quality and shading effect (Green water culture).
- The tank culture of algae has to be equipped with aeration on some points for fertilizer turbulence.
- The initial culture of microalgae (inoculums) has to be taken from the intermediate laboratory culture and then cultured in all the tanks gradually until the sufficient algal mass is achieved.
- It is necessary to sterilize the water using chlorine 10 ppm for 24 hours, then to neutralize by using Sodium Thiosulfate 5 ppm. To enrich fertilizers, several agricultural fertilizers can be used as shown in the Table below

Fertilizer	Concentration (ppm) / tonns
Urea	40-60
Ammonium sulphate	20-40
Ammonium phosphate	20-30
EDTA	1-5
$FeCl_3$	1

(or)

Ammonium sulphate	100 g
Ammonium phosphate	10 g
Urea	10 g
Ratio - (1:1:1)	

For the mass culture, an early density usually have 2-3 million cells/ml.

- If the sunlight is normal, the algae will grow and will be ready for use after 4-5 days, with the density of 12-15 million cells/ml.
- *Microalgae* should be harvested before it reaches the peak of growth or when the algae are on stationary phase.
- Algae can be directly pumped to the destination tank such as algae seed tank, rotifer culture tank, copepod tank and larval rearing tank.
- The rest of the microalgae in a mass culture tank can be used for re-culture by adding filtered seawater and fertilizer. This method can be employed for several repeated times (2 – 3 times).
- After that microalgae in the culture tank is totally harvested and the tank needs to be cleaned to prepare for a new culture.
- For a medium size fish hatchery, several tanks are required to culture phytoplankton so that it can be harvested every day by turns.

No.	Parameters	Range
1.	Salinity	30 – 33 ppt
2.	Temperature	26 – 31 ° C
3.	Light	12 L : 12D
4.	pH	8.1 – 8.3
5.	Dissolved Oxygen	4.5 – 6.5 ml / lit

Copepod culture

Copepods constitute a first vital link in the marine food chain leading from primary producers to fish. In the open water marine environment, calanoids dominate the herbivorous zooplankton and provide the food chain base for practically all marine fish larvae and planktivorous fish.

Free living copepods have generally cylindrical bodies with a narrow abdomen (planktonic forms) or in the case of benthic or surface living forms, broader bodies and/or dorsoventrally compressed forms. The trunk is composed of a thorax (metasome) and an abdomen (urosome). The head (cephalosome) is fused with the thorax and bears anteriorly a typical median naupliar eye, a conspicuous set of antennae and the various appendages used for feeding and swimming. The anterior part has been designated as the prosome. The abdomen is generally narrower than the thorax, with no appendages except for the caudal rami. The genital opening is usually located in the first abdominal segment, whereas the last segment bears the anal opening (anal somite).

The urosome ends in a furca formed of two symmetrical rami ornamented with setae. The antennules vary in size and may be used to distinguish among the genera –shortest in harpacticoids and longest in calanoids. The oral aperture is situated ventrally and it is surrounded by mandibles, which help to macerate the food, maxillules adapted to grasp and break up the food and maxillae, which adopt different forms according to the feeding mode of the species and help in capturing the food. In filter feeders, the maxillae bear setae where particles are retained, accumulated and transferred to the mouth. Attached to the thorax are four or five pairs of swimming legs, essential for locomotion. In calanoids and some harpacticoids, the last pair is often modified, being often reduced in females and enlarged and asymmetrical in males and used to grasp the female during mating. The body of the newly hatched nauplii has an oval shape, is dorsoventrally compressed and unsegmented,

with relatively few appendages and a single naupliar eye. During the remaining three naupliar stages, there is a progressive development of setae and of appendages in the posterior end of the body.

Due to the rapid expansion of hatchery production of seeds for marine aquaculture and increasing interest in new species. There is a requirement which cnnot be met by conventional species of live feeds such as rotifer and a *Artemia*. Thus interest in copepods has been generated and the use of copepods as live feed in aquaculture is rapidly gaining impetus. They are employed mainly because they are the only acceptably sized prey for many species of marine finfish. The most commonly used species in aquaculture are free living copepods belonging to two orders – Calanoida and Harpacticoida.

The calanoids are predominantly pelagic, occurring at all depths, with some near-bottom and benthic species. They are selective feeders feeding on small phytoplankton cells by filtration or predators feeding on a variety of animal prey including copepod eggs. They are distinguished by their long antennules, as long as the body itself or even longer, with upto twenty seven segments and biramous antennae used as accessory locomotory appendages. The position of the prosome-urosome articulation is between the fifth and sixth post cephalosome somite. The harpacticoids which include over 50% of copepod species are primarily marine, free living, benthic organisms. They inhabit sediments occupying spaces between sand particles (interstitial), burrowing into sediment (burrowers) or living on sediment or plant surfaces (epibenthic). They are distinguished by their short antennules, fewer than ten segments, and biramous antennae. The position of the prosome-urosome articulation is between the fourth and fifth post cephalosome segment.

Reproduction

Most copepods reproduce sexually. The male deposits a sac containing viable sperm called a spermatophore near the genital

aperture of the female. Most calanoids are broadcasters, shedding eggs singly into the water. The number of eggs spawned in a single event may vary from a few eggs to 50 or more eggs, and each spawning event may occur once every 24 hour for extended periods. Free spawning species such as various *Acartia* species may produce between 11 and 50 eggs per female per day, producing a total of more than 1200 from one single spawning and Calanus species between 15 and 230 eggs per female per day to a total of upto 3800 eggs per female. In most cases, a new mating is necessary for a female to produce eggs again. Other copepods including cyclopoids and harpacticoids, have their eggs contained within one or two egg sacs (ovisac), which remain attached to the female genital segment until they hatch. In calanoids, the eggs are not contained in a membrane but adhere to each other as an egg mass and remain attached to the female. Each egg sac or egg mass may contain a few to 50 or more eggs and may be produced at frequent intervals of a few days. Egg production is measured as number of eggs per female per day. Resting eggs are produced by several species of copepod and are the primary mode of dormant state in calanoids. These copepod eggs are laid during development, whereby development is arrested, and possess an additional external envelope of variable thickness. Resting eggs are able to withstand long periods of desiccation, heat or cold. Resting eggs have only been reported for calanoids. Day length and temperature were the principal environmental cues that induce females to switch from active reproductive to a resting reproductive state.

Life Cycle

Once fertilised the eggs pass into the water or into an egg sac. The egg is spherical and protected by a chitinous envelope. The larva that hatches from copepod eggs, the nauplius (NI), develops through five or six moults before passing onto the copepodite stage where they display the general adult features. Most species

commence feeding during the third or fourth naupliar stages, although a few species commence feeding during the second and even fewer during the first naupliar stage.

Size

Calanoid eggs produced in egg sacs range in diameter from 70 to around 800µm and are generally larger than freely spawned eggs, which measure around 200µm. Size ranges of newly hatched nauplii also vary. Newly hatched nauplii such as *A. tonsa* measure less than 100µm in body length whereas as the larger calanoid nauplii measure around 220µm.

Generation time

The generation time defined as the time interval between hatching of an individual and the hatching of its progeny which differs from species to species and is positively correlated with increasing temperature. In calanoids reared at different temperatures, generation times varied from around one week in *Acartia* spp to months. Food supply and salinity may also influence development rates.

Feeding, Food quality and Food availability

Calanoids are generally herbivorous filter feeders, able to distinguish between particles and selecting between different food particles based on size or taste. The rate of consumption of algal particles is influenced by the size, quantity and quality of the food. However, few species of calanoids have a non-visual, active raptorial mode of feeding, capturing and ingesting a variety of animal prey. Copepod species may also create feeding currents that entrap non-evasive prey. The food concentrations at which egg production commences and at which it attains a maximum level differ between species. Egg production per female per day increases with increasing food concentrations to an asymptotic level. Food quality also influences growth and reproduction.

Harpacticoids are primarily detritivorous, benthic grazers, efficiently utilizing various food sources such as bacteria, microalgae, marsh grass, algal biofilm, diatoms, polychaete meat, etc. Although harpacticoids eat practically anything, their offspring production is not independent of the food quality. Food supply in terms of quality and quantity affects feeding, development and reproduction in harpacticoids.

Production Methods

(i) Production in enclosed sea areas

Potential predators in small enclosed areas are initially to be eraticated. The phytoplankton production is enhanced by adding agricultural fertilizers. In some cases, copepod starting cultures are derived from the resting eggs in the sediment. In other cases, wild zooplankton are collected from the sea and transferred to the enclosures.

(ii) Production in outdoor ponds or large tanks

Outdoor production in 350- 5000 m^3 ponds and tanks are being carried out. Filtered seawater is generally used in this system. By using filters of around 20-40 µm, natural phytoplankton can be transferred to ponds without the accompanying zooplankton or potential predators. The phytoplankton can be fertilized to induced blooms. Filtering devices for selective sieving are used to collect primarily nauplii (80-250 µm), copepodite stages (80-350 µm) or primarily adult stages (250–600 µm) to inoculate the rearing tanks. In Asian countries, copepodites and adult stages of copepods were added in tanks 3 days before stocking of the newly hatched fish larvae. In this system, using wild harvested copepods *(Acartia* spp, *Pseudodiaptomus* spp., *Oithona* spp.,and a few harpacticoids) an average survival of 3.4% at harvest of *Epinephelus coiodies* is obtained.

Regular monitoring of densities of the live prey in these outdoor

systems is important for the successful rearing of marine fish larvae.

(iii) Intensive Culture

Required for rearing species with very small larvae, or for species difficult to rear with traditional prey or for species with small mouth such as grouper and red snapper.

Calanoids

Most frequently cultured are coastal species such as *Acartia, Centropages, Eutemora* and *Temora*. They are small, relatively of short generation times and a wide thermal and salinity tolerance and are easily adaptable to laboratory condition. Most calanoids require phytoplankton. In many cases the copepods are reared on monoalgal diets, which may not comply with all the requirements for maximum egg production. Somatic growth ceases in adult copepods and growth rate is more or less equivalent to the rate of egg production. The rate of egg production in copepods is dependent on the size, quantity and quality of the algae provided. As a general rule to reach food saturation, high ingestion rates and high egg production rates, cell concentrations of around 10^3 cells ml^{-1} would be sufficient using larger cells and around 10^4 cells or 10^5 cells per ml using smaller cells. A combination of at least two algal species with high n-3 polyunsaturated lipid content, and of a size that can be utilized by both the feeding naupliar stages and the copepodite and adult stages comprises an adequate diet for culture.

In one of the culture systems, the eggs are sedimented to the bottom from where they were siphoned daily, simultaneously siphoning out debris, faecal matter and associated ciliates. During the siphoning, the eggs were concentrated on 45µm sieve, allowing most of the debris and ciliates to pass through and removed from culture. The daily removal of eggs eliminates the potential loss of nauplii through cannibalism by adult population. The removed debris was checked daily, together with egg count. The presence of

ciliates (*Euplotes* sp.) heralds the deterioration of water quality and a thorough water exchange was necessary. Failing to do this will result in a culture crash. The culture was filtered through a 180µm sieve submerged in sea water to retain the adult population and wash out the ciliates. The adults were then used to inoculate a new tank filled with filtered (1µm) sea water. The frequency of this water exchange varied, but was generally done around every 2-4 months.

Batch culture

In a reliable batch culture system for rearing *Acartia* sp., consistent production results over an 8 day cycle in three 1000 litre tanks run concurrently over a period of 7 weeks. Starting with an inoculum of around 50-100 adults and 150 to 250 copepodites per litre, the culture contained after 7 days around 2000 nauplii, 750 copepodites and 300 adults per litre.

Light

A photoperiod of 12 hour of light is sufficient. High solar radiation is harmful to copepods; hence adults show negative phototaxis during the day and positive phototaxis during the night.

Aeration and Oxygen

Aeration is required to maintain phytoplankton in suspension and create small turbulence, which helps to distribute the copepods. Too vigorous aeration should be avoided and unnecessary.

Culture tank size and shape

Most calanids require large volumes and the adult density rarely exceeds 100 per litre. Cylindrical tanks with flat bottom (200 litres) are suitable for culture of copepod, *Acartia tonsa.*

Temperature and Salinity

Temperature has a vital role for copepods. But their

adaptability to temperature is remarkable. Coastal species have wider thermal and salinity tolerances than oceanic species.

Contamination

Contamination of copepod cultures by bacterial blooms, ciliate infections, other copepods or rotifers may pose a problem. Use of same siphon/sampling devices, etc. for all copepod tanks should be avoided. In commercial facilities, contamination by rotifers is the most likely cause of the collapse of copepod culture, since the rotifers with their higher reproductive rate would quickly outcompete the copepods. It is therefore important to keep these cultures strictly apart.

Ciliates are utilized by copepods and may in periods of low phytoplankton concentrations constitute the major dietary source. In intensive cultures, the presence of certain ciliates such as *Euplotes* sp. is often an indication of overfeeding and should be avoided. For eliminating ciliates it is advisable to empty the culture using a 60 or 80µm mesh which retains the adult copepods, but allows the ciliates to be washed out. Cultures may succumb to uncontrolled proliferation of bacteria, Eventhough bacteria often constitute a part of the diet of copepods. Calanoids are sensitive to high ammonia concentrations.

Harvest and Storage

Copepods can survive for short periods in gauze as they are transferred from one tank to another. They can also survive for an extended time at very high densities, provided that there is sufficient oxygen. Freely spawned calanoid eggs sink to the bottom and can be harvested by siphoning the bottom once daily. The day's production can be transferred to individual hatching tanks to be used as live feed.

Harpacticoids

Harpacticoids have been cultured in batch and continuous

systems to provided food for marine fish larvae and several studies have demonstrated improvements in growth and survival when using harpacticoids.

Advantages

(i) High tolerance to a wide range of environmental conditions

(ii) Ability to feed on a wide range of live or inert diets

(iii) High reproductive capacity

(iv) Relatively short life cycles (7-29 days)

(v) Ability to be cultured in high densities

(vi) Requirement for surface area rather than volume

(vii) Planktonic naupliar stages

(viii) Can be used as tank cleaners in rotifer cultures, other copepod cultures or larval tanks.

The culture conditions for harpacticoids are less demanding than those for calanoids. Filtered seawater may be used and a whole range of inert food is acceptable to many harpacticoid species. This simplifies the culture method and eliminates the need for culture of phytoplankton.

Food and feeding: If algae are readily available, a mixture of two algal species would be the preferred choice. Algae which quickly sediment are very appropriate for benthic copepods, possibly because bacteria colonise these cells, and the mixture of algae and bacteria may be a superior combination for harpacticoids.

Light: A photoperiod of 12L/12D was shown to be most favourable for offspring production.

Aeration: Aeration may be applied to maintain an even distribution of food

Culture tank size and shape: The mass culture of benthic harpacticoids is dependent on the availability of surface area rather

than culture volume.

Temperature and Salinity: Most harpacticoids have wide thermal and salinity tolerances.

Contaminants: Rotifers and ciliates are the major contaminants

Harvest, storage and Transport

Since harpacticoids are not free spawners, harvest methods for collecting nauplii need to be developed. Concentrating nauplii by light can be practised for harvesting. Harpacticoids are relatively tolerant to high stocking densities and can be transported for a period of upto 2-3 days, kept cool in blood transfusion bags (2 litres) at densities of 200,000 individuals per litre. Excess nauplii can also be stored at 4°C for upto one week and used on days when production output is below the required amount.

Nutritional value for Fish Larvae

Improved growth, survival and/or rates of normal pigmentation have been documented for several marine fish species fed copepods alone or as supplement to the traditional diets of rotifers or *Artemia* nauplii compared with traditional diets alone. In many hatcheries, malpigmentation of the reared juveniles constitutes a major problem. Larval nutrition is suggested to be the major factor determining pigmentation patterns. The documented improvements in larval growth, survival and rates of normal pigmentation are generally attributed to levels of DHA, EPA and/or arachidonic acid (ARA) in the diet. DHA can be synthesized from shorter chain precursors in some marine fish larvae, but at rates insufficient to meet requirements for their normal growth and survival. A minimum of 0.5 to 1.0% of dry weight as n-3 HUFA is required for juvenile marine fish and higher amounts are required for rapidly growing fish larvae.

Marine copepods, the principal diet for most marine fish larvae in nature, contain high levels of DHA and other PUFA, either obtained through their phytoplankton diet or accumulated despite low PUFA levels in the diet. DHA levels in wild copepods can be more than 10 times higher than in enriched *Artemia*. DHA is important in maintaining structural and functional integrity in fish cell membranes, in neural development and function, and especially in retinal development and vision. It is suggested to play an important role in the development of normal pigmentation when provided in sufficient quantities at particular times during the larval stage. EPA cannot be synthesized by most marine fish and it is therefore essential in the diet of the fish. EPA gives rise to less biologically active eicosanoids than those produced from ARA. Since it competes metabolically for the same enzyme systems required for ARA derived eicosanoid production.

EPA is very important in modulating the production of these highly biologically active eicosanoids. This metabolic interaction necessitates an optimal EPA: ARA ratio in the diet. Eicosanoids of n-6 origin are important for the normal function of vital organs such as kidney, gill, intestine and ovaries of marine fish. Levels of ARA in copepods are high in both calanoids and harpacticoids. Apart from the superior fatty acid composition in copepods, they contain high amounts of polar lipids. Polar lipids are more easily digested by larvae and may also facilitate digestion of other lipids in the undeveloped gut of marine fish larvae. Varying concentrations of the carotenoid astaxanthin were found in the various copepods and its possible value for fish is as a precursor to Vitamin A. Copepods are also an important source of exogenous digestive enzymes and are thought to play an important role in fish larval digestion.

The ability to culture copepods on a mass scale with high density is the major bottleneck for their utilisation in the seed production of marine finfishes. It has been noted that feeding with mixed culture of suitable sized rotifers is advantageous for the

survival of larvae since a variety of size ranges of nauplii will be available as larval feed. The copepods selected for mixed culture *viz.* the calanoid *P.serricaudatus* and the harpacticoid *E.acutifrons* have the required size nauplii suited for the initial feeding of the larvae. The adult *P.serricaudatus* occupied the water column of the culture tank whereas the adult *E.acutifrons* was mostly at the bottom of the tank. But the naupliar stages of both the species were spread throughout the water column which facilitated larval feeding. However maintaining a high density mass culture of copepods similar to rotifer culture is not easy since the multiplication rate of copepods cannot be compared to rotifers. The low density of copepods in the mixed culture experiments is indicates the same.

Harpacticoid (*Euterpina acutifrons)*

Body variable in shape. A1 short, prehensile in male. A2 Exp well-developed. Md with biramous palp. P1 not prehensile, similar to P2; all with 3-segmented Expand Enp. P5 lamelliform, 2-segmented. Ovisac simple.

Prosome of 4 somites. Its postero-lateral corners flattened and prolonged into points. Urosome of 5 somites in female and of 6 somites in male. Caudal rami short, setae very short. A1 7-8-segmented, with short setae and long sensory organs; male A1 prehensile on both sides. A2 3-segmented; exopod rudimentary, represented by 1-2 setae. Mxp 2- segmented, elongate; with terminal claw; male differs from female. P1 Enp 1-segmented, exopod 3-segmented. P2-4 with both rami 3-segmented. P5 rudimentary, with 2 long segments; symmetrical or almost in male.

Free living, benthic organisms.

Inhabit sediments occupying spaces between sand particles, burrowing into sediment or living on sediment. High tolerance to a wide range of environmental conditions

Harpacticoid copepods are favoured over calanoids, since

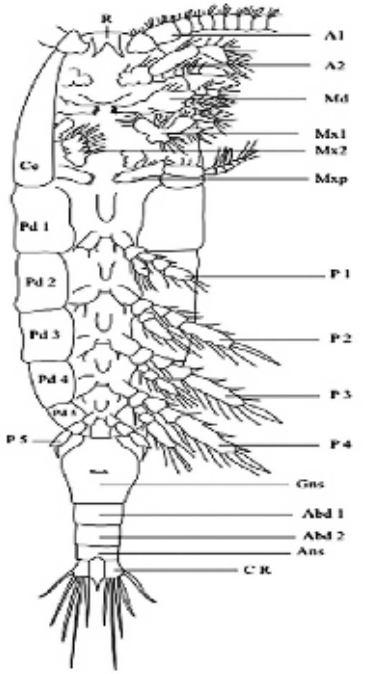
harpacticoids, as a result of their benthic habitat, can be reared at much higher densities. However, their benthic nature also makes mass culture difficult, since large surface areas must be provided.

Harpacticoids are primarily detritivorous, benthic grazers, efficiently utilizing various food sources such as micro algae, algal bio-film and diatoms.

Harpacticoids are smaller and range in size from 40 micron (Nauplii -1) to 800 micron (adult), short life cycles (7-29 days) Planktonic naupliar stages

The generation time is defined as the time interval between hatching of an individual and the hatching of its progeny – varies with temperature.

The different parts of an adult copepod



Female

Body fusiform. Caudal rami and anal somite of approximately equal length. P1 basis with teeth along distal edge. Cephalosome and Pd1 fused. Prosome of 4 somites; rostrum uniramous and stout. Urosome of 6 somites. A1 7- segmented. A2 Exp well-developed, 3-segmented. Mdp Exp and Enp 1-segmented. P1 Exp and Enp 2-segmented, P2-P4 Exp and Enp 3-segmented. P5 symmetrical, lamelliform, 1-segmented, with 4 apical setae.

Male

A1 geniculate on both sides, with segments 4 and 5 fused and much thickened; segments 6 and 7 fused. P5 symmetrical, rudimentary; each ramus furnished with 2 apical and 2 outer setae.

Life cycle

- The male deposits a sac containing viable sperm called a spermatophore near the genital aperture of the female.
- Harpacticoids have their eggs contained within one or two egg sacs which remains attached to the female genital segment until they hatch.
- Egg production is measured as number of eggs per female per day.
- Fecundity in species with egg masses is within the range 18 -20 eggs .
- Fertilized eggs pass into the water or into an egg sac.
- The egg is spherical and protected by a chitinous envelope.
- The larvae that hatched from copepod eggs, the nauplius (NI) develops through 5 or 6 moults before passing into the copepodite stage where they display the general adult factures.
- Harpacticoid nauplii feed from the 1st N stage and undergo six moults, the final one resulting in the first copepodite stage.

Isolation and Identification

Collection from the wild – filter with particular mesh size (100micro to 500 micron)

100 nos individual (1:1 M/ F) isolate to 50 ml test tube

Add microalgae as feed (5 ml / 2 days once)

Provide light period 12L / 12 D; Salinity: 27 to 35; Temperature: 28 to 31^0C

Generation time – 7 days

Harvest nauplii / copepodite (harvest continuously for 3 months)

Intermediate culture

Nauplii (1000 nos) transfer to 1000 ml beaker

Add microalge as feed (25 ml / 2 days)

Provide light period 12L / 12 D; Salinity: 27 to 35; Temperature: 28 to 31^0C

Generation time – 7 days

Collect nauplii / copepodite

Mass culture

Nauplii transfer to 1 to 2 tonn tank

Add microalgae as feed (500 lit algae / week)

Provide light period 12L / 12 D; Salinity: 27 to 35; Temperature: 28 to 31^0C

Generation time – 7 days

Harvest nauplii / copepodite (3-5 nos / ml)

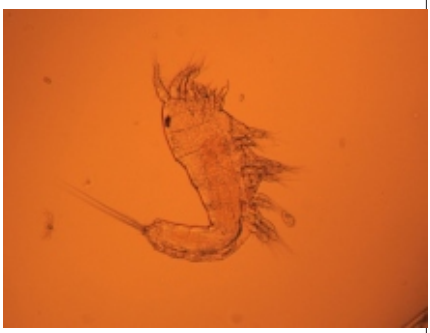
Total drain and harvest the tank after 1 month

Culture Protocol

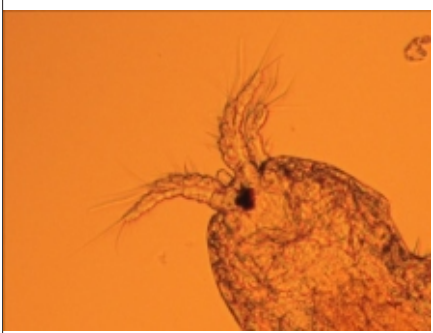
- *Population counts should be done weekly for feeding adjustments.*

- *Count algae concentrations and compute required feed volumes to be added.*
- *Siphon detritus from tank bottom daily.*
- *Remove sufficient water volume to allow addition of new feed volume(s), while maintaining 200 L total volume. As the population matures this volume can be increased to 300 L. Adjust feed computations accordingly.*
- *Use 40 micron will retain faeces, nauplii, and eggs. Rinse sieve gently into separate collection bucket. Buckets are allowed to stand for 10 minutes to allow settling. Adults and nauplii are then attracted to the upper layer of the bucket with light and decanted for return to the tank.*

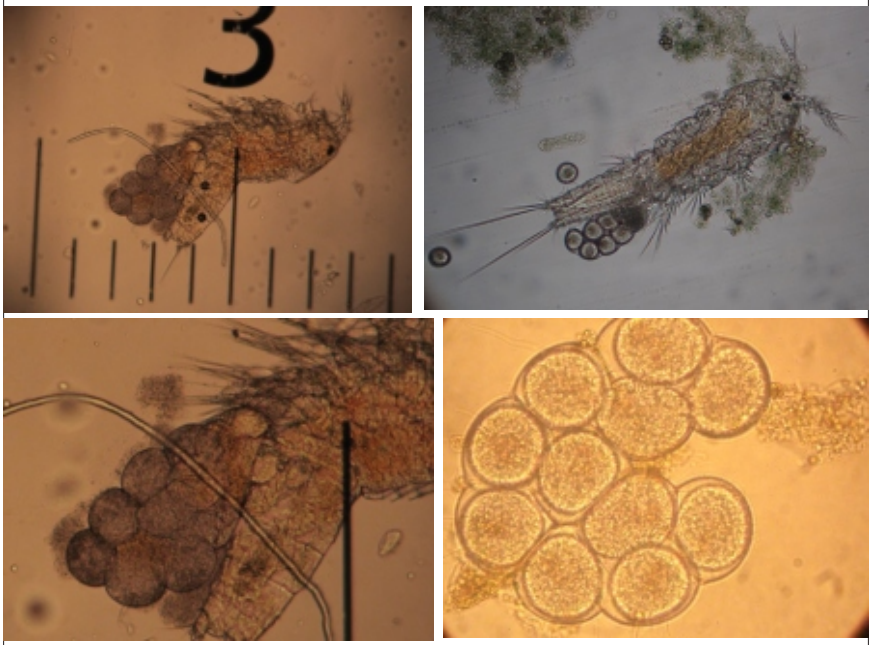
Images of *Euterpina acutifrons*



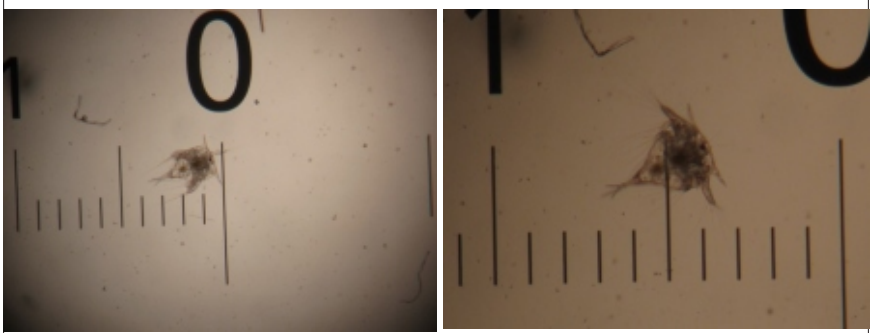
Anterior & Posterior view



Berried Copepod



Harpacticoid nauplii



Advantages of Copepods

The three main copepod orders *viz.,* Calanoida, Harpacticoida and Cyclopoida have been investigated for their suitability as feeds for larval and juvenile fish. While each copepod order has its advantages and disadvantages, it is generally agreed that the following are the benefits of using copepods for larviculture.

(i) Copepods have a larger size range from first nauplii to adult copepodites and offer good size ranges for the entire hatchery phases for certain species of finfish

(ii) They have superior nutritive value in comparison to rotifers and *Artemia*

(iii) Copepod nauplii may be more easily and completely digested than either rotifers or *Artemia*

(iv) Copepods are natural sources of antioxidant astaxanthin and Vitamins C and E

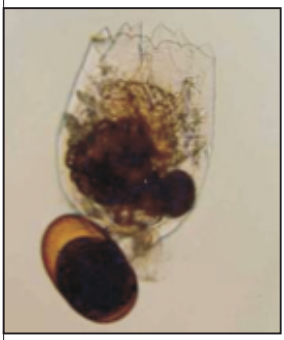
(v) The movement of copepods and their nauplii triggers the feeding responses in fish larvae. The 'jerking' swimming action of most copepod nauplii and adults is an important stimulus for initiating feeding by fish larvae

(vi) Use of copepods in larval fish diets have been associated with a decrease in fish malpigmentation and deformity rates

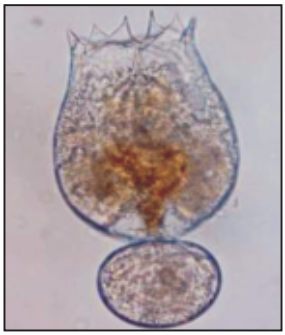
Rotifer culture

The rotifers, *Brachionus plicatilis* and *B. rotundiformis* are widely employed for feeding marine fish larvae. Its body size ranging from 70 - 350 microns, (depending on strain) makes these organisms an appropriate prey to start feeding after the resorption of the yolk. Rotifers are used as first food during a few days or weeks depending on the reared species. The main advantages of rotifers include – 1). High population growth rate, 2). Filtration of particles in suspension, 3). A good tolerance to culture conditions and handling, and 4). Appropriate energy content and reasonable nutritional value. In addition, it is relatively modifiable by dietary manipulation by means of post-culture enrichment.

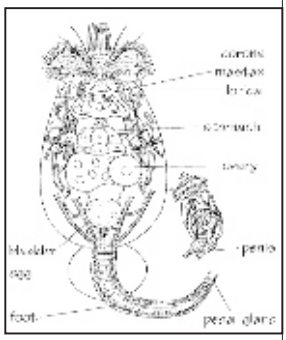
Rotifers are the smaller size zooplanktons widely used in marine fin fish hatchery operations. The marine fin fish larvae initially feeds on the such smaller size zooplanktons and hence suitable size of rotifers need to be cultured in mass to feed the fish larvae. The important criteria for selecting the rotifer depends on the



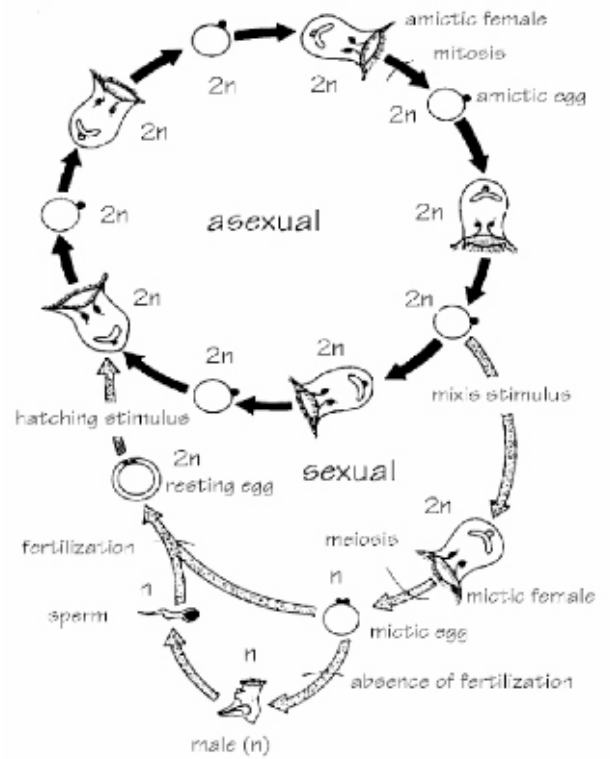
Brachionus plicatilis



Brachionus rotundiformis



Morphology



mouth size of the fish larvae, digestibility, nutritive value of the rotifer and easy for culture and proliferation. Marine and brackish water rotifer species can be artificially propagated in seawater and more popular rotifer species used for marine fin fish hatcheries are

Brachionus plicatilis and *Brachionus rotundiformis.*

Based on the length of lorica, *Brachionus* is separated into 3 strains,

B. plicatilis as L type (large) with long of lorica 200 – 360 µm

B. rotundiformis as S type (small) with long of lorica 150 – 220 µm

B. rotundiformis as SS type (super small) with long of lorica 70 – 160 µm.

WHAT ARE THE ADVANTAGES OF ROTIFER AS LIVE FEED?

1. Small body size and round shape
2. Slow swimming speed and habit of staying suspended in the water column
3. Easily enriched with external nutrients resources
4. High reproduction rate and high density cultures
5. Very rich In low molecular weight water soluble proteins
6. Contain a broad spectrum of digestive enzymes such as proteases, peptidases, amylases, lipases and even celluloses

Pure culture of Rotifer

- Incubated in 500 ml *erlenmeyer* containing sterile sea water. The culture condition should be equipped with *flourescent* lamp and aeration to supply oxygen.
- Microalgae such as *Chlorella sp, Tetraselmis sp, Dunaliella* and *Isocrysis sp* can be used as feed for rotifer.

Mass culture of Rotifer

- Outdoor mass culture can be carried out in volume range of 5 – 12 m^3 (1 tonne, 2 tonne, 5 tonne, 10 tonne)
- Phytoplankton has to be added to rotifer culture tank with density 3-4 million cell/ml for starting the rotifer culture. Rotifers have to be inoculated into tank with initial density 50-100 rotifers/ml

- The next day, algae has to be added into rotifer culture tank
- Practically, growth of rotifer can be identified by change of water colour into transparent

No.	Parameters	Range
1.	Salinity	27 – 33 ppt
2.	Temperature	26 – 31 ° C
3.	Light	2,000 – 3,000 lux
4.	pH	7.5 – 8.3
5.	Dissolved Oxygen	3 – 6.5 ppm

Continuous Culture

- Rotifers can be harvested by flowing out culture water through a plastic hosepipe into a rotifer sieving bag with mesh size of 60micron at the end point of those hosepipe
- Collected rotifers can be transferred into bucket for enrichment with different types of enrichment media.
- Every day, around 30% volume of rotifer culture tank has to be harvested from the total volume of tank
- After repeated use of 3 weeks, the rotifer culture tank should be totally cleaned for fresh culture

Rotifer enrichment

- Rotifers can be enriched with Highly Unsaturated Fatty Acids (HUFA's) which contains high levels of the essential Omega-3 fatty acids, Eicosapentaenoic acid (EPA) Docosahexaenoic acid (DHA).
- HUFAs are important to maintain fluidity of blood in blood vessel under low temperature condition for the fish larvae
- Especially DHA, an essential fatty acid that accumulates in the brain of fish during early development and it increases neural

function

- Feeding with DHA-enriched diet at an early stage of fish larvae has been successful in improving pigmentation
- EFA deficiency creates syndromes like poor vitality, poor growth, low survival and death to simple stress (shock).

Enrichment methods

- Indirect method through feed medium like *Nannochloropsis* and ω – yeast. Rotifers in each culture tank can be fed with concentrated microalgae of *Nannochloropsis* ($1x10^4$) every 8 hrs and yeast once in a day at morning.
- Direct method with emulsified oil (fish oil, etc) for 100 million rotifer with fish oil ± 5 ml, raw egg yolk ± 2 ml and tap water 100 – 200 ml

Enrichment with scot emulsion

- Harvested rotifer has to be transferred into a 35 lit tub containing 3/4 part of *Nannochloropsis* medium and 1/4 part of rotifer (600 to 800 nos / ml)
- 1-5 ml scots emulsion oil can be mixed with freshwater and stired well.
- This emulsion can be added into the tub containing rotifer and sea water.
- Strong aeration has to be provided for 2 hours.
- Harvest rotifers and can be fed to the fish larvae (10 to 12 nos / ml of rotifers).

INVE A1 DHA and SELCO INVE- SELCO (Self Emulsifying Liquid Concentrates)

- Four 2 ton tanks of rotifers are harvested daily (three days after stocking) at a density of 600-800 rotifers/ml.
- Enrichment is done in buckets containing a volume of 40 L with a

density of 800-1000 rotifers/ml. Approximately 10 lit of *N. oculata* (1.0x10^{6}cells ml) are added.

- Buckets receive aeration to maintain DO levels above saturation.
- Enrichment media (INVE DHA selco) at doses of 0.3 g per 1x10^{6} rotifers were added at 08.30 and 18.30 hours and rotifers are allowed to feed for 4-6h at 27-28 C.
- Once enrichment is completed, rotifers are filtered to remove residual enrichment diet and placed into a clean bucket containing a final volume of 35 L.
- Enriched rotifers at a density of 10-12 nos. per ml were fed to 3 to 9 dph larvae.

Production of the brine shrimp *Artemia* nauplii

It is used in marine aquaculture worldwide. Although Artemia is not a natural part of marine larvae, it has been favoured due to its convenience for use and high nutritional value. One of its most interesting features of this organisms is its ability to form dormant cysts that are highly resistant to adverse environmental conditions and can be kept viable for years. They are normally stored under dry and cool conditions. The ease and simplicity of hatching brine shrimp nauplii makes them the most convenient and least labour intensive live food available for aquaculture. However, the only negative aspect is its high cost, hence now a days the practical strategy adopted in larviculture of marine species has been to attempt early weaning in conjunction with a prolonged rotifer feeding period to eliminate the use of Artemia. However, this is not always possible and in some species whose larvae are relatively larger at hatching, Artemia nauplii might even be the only live prey used in larviculture.

***Artemia* cyst strains**

Having a larger size than rotifers, the brine shrimp *Artemia salina* are used as the second (after rotifers), live feed organisms to

feed fish larvae. *Artemia* is not cultivated in the hatchery as in the case for algae and rotifers, but their larval stages are obtained by incubating and hatching their resting eggs, which are available commercially as dry storable cysts. The first *Artemia* larval form is the nauplii, which are smaller in size and richest in yolk, and followed by a larger size metanauplius, whose nutritional value has to be boosted by feeding them with special enrichment diets 12 to 24 hours before feeding to the fish larvae.

Cysts of different strains can be used as per the requirement:-

(i) Cysts giving small Instar I-nauplii (with a length of around 430 μm at yolk- sac stage) with high levels of the essential highly unsaturated fatty acids (n-3 HUFA). An example is the AF strain of the Belgian producer INVE. Such nauplii allow an early switch from rotifers to Artemia in larval feeding.

(ii) Cysts producing medium-sized Instar I-nauplii (around 480 μm) with high levels of n-3 HUFA, such as the AF 480 strain of INVE. These nauplii are useful to switch from the smaller nauplii to the bigger metanauplii.

(iii) Cysts hatching large Instar I-nauplii (around 520 μm) with low levels of n-3 HUFA, such as the EG strain of INVE. These cysts, which are the commonest and cheaper, are widely used to produce metanauplii which represent the bulk of live feeds in larval fish rearing.

Their enrichment is mandatory to increase the essential fatty acids content to meet the nutritional requirements of young fish. Two additional parameters characterize the *Artemia* batches: the number of cyst per gram and their hatching rate (the number of nauplii produced per gram of cysts). The best strains can give about 290 000 - 300 000 nauplii per gram of cyst hatched, with a hatching rate close to 95%. In a hatchery the use of good quality cysts allows a synchronization of the production cycle on a 24-h period, with the harvest of freshly hatched nauplii coinciding with the start of the

incubation of new batches.

Disinfection and decapsulation of brine shrimp cysts

Artemia cyst shells are usually contaminated with bacteria, spores of fungi and other microorganisms. Fish larvae can be infected when untreated empty shells, unhatched cysts or cyst hatching medium residues are introduced into the larval rearing tank. Before incubation, cysts should therefore be disinfected. This process also improves hatching by reducing the bacterial load of the hatching medium. Disinfection is done by keeping the cysts for a few minutes in a hypochlorite solution at a maximum density of 50 g/litre. This product is easily available as commercial grade bleach. The duration of the treatment varies according to the active chlorine concentration of the disinfecting solution.

Typical duration is :

- 1 minute in a 10 000 ppm solution,
- 20 minutes in a 200 ppm solution.

As in commercial bleach, the chlorine content may range from 5 to 15%, it is mandatory to check the actual chlorine concentration in the bleach that is going to be used. This can be done either by titration or by determination of the refractive index. The following example shows how to disinfect one kg of cysts in a 200 ppm hypochlorite solution obtained from a household bleach with 5% active chlorine:

- One kg of cysts needs 20 l of fresh water for the disinfecting solution.
- If this solution is going to be used for a 20 minutes bath you will need 20 l x 200 mg/l = 4 000 mg = 4 g active chlorine.
- The quantity of 5% bleach required to give 4 g active chlorine is: (1 000/50) x 4 = 80 ml pour 80 ml of 5% bleach in 20 l of fresh water.

- Add one kg of cysts; place an airstone for continuous aeration to keep cysts in suspension, and keep the cyst in the solution for 20 minutes.
- Harvest cysts on a sieve (125 ìm mesh size) and rinse thoroughly with plenty of tap water.
- Transfer the rinsed cysts to the incubation tank.

A more effective way to obtain completely contaminant-free cysts is decapsulation, which implies the elimination of the cysts thick external layer, the chorion, by chemical oxidation. This process, which requires greater attention, has additional advantages. As they spend less energy to hatch after the removal of the chorion, the hatching nauplii have better nutritional value. Moreover, fish do not risk suffocating by gulping empty or unhatched cysts offered together with the nauplii, as it may happen when using disinfected cysts. The decapsulation process consists in four steps: hydration, treatment in a chlorine solution, washing and deactivation of the residual chlorine. The example described below refers to the decapsulation procedure of one kg of cysts. The hydration, a necessary step as the complete removal of the chlorine may only happen when cysts are spherical, proceeds as follows:

- Water volume required: around 6 l per kg (maximum amount: 200 g/l); both fresh and sea water can be used; water temperature should be between 20-25°C;duration: 45 minutes;
- aeration: sufficiently strong to keep cysts in constant suspension; use an open end pipe in a 10l bucket.
- Collect the hydrated cysts on a sieve and treat them immediately with the decapsulation solution.

The decapsulation solution requires a source of hypochlorite, usually liquid bleach (NaOCl), and an alkaline product to increase pH level of the decapsulation solution above pH10. Usually technical grade caustic soda (sodium hydroxide

NaOH) is used. The first product is added at 0.5 g active chlorine per gram of cysts, and the second as 0.15 g of sodium hydroxide per gram of cysts. For hydrated cysts the procedure is as follows (figures refers to one kg of cysts):

- Prepare 0.5 g Cl x 1 000 g cysts = 500 g of active chlorine, equal to 3 33 l of a 15% bleach.
- Prepare 0.15 g NaOH x 1 000 g cysts = 150 g of NaOH, equal to 0.375 l of a 40% NaOH solution.
- Put the bleach and NaOH in a suitable container (e.g.: a 20 l plastic bucket) and fill with seawater to 14 litres (14 – 3.33 – 0.375 = about 10.3 l of seawater) provide a strong aeration and eventually if available add antifoam.
- Place the hydrated cysts in the bucket; control the temperature: it should remain within 25°-30°C. In case of higher temperatures, add ice to prevent that it reaches 40°C which are lethal for the cysts.
- Verify cyst colour changes. The change in cyst colour confirms that decapsulation is in progress.
- The cyst colour shifts from dark brown to grey and finally to orange, which is the colour of the nauplius body seen by transparency through its outer cuticular membrane, left exposed by the dissolution of the chorion. The process usually lasts 5 to 15 minutes.
- Using a pipette or a graduated cylinder, check floatability: non decapsulated cysts will float and decapsulated cysts will sink; as soon as all cysts have turned orange, stop the process by harvesting them on a sieve and rinse thoroughly with plenty of tap water and rinse well until no more chlorine smell is noticed
- The residual hypochlorite adsorbed by the decapsulated cysts has to be neutralised by dipping them in a 0.1% solution of sodium thiosulfate ($Na_2S_2O_3 . 5H_2O$) for 5 minutes; then, after a

final rinsing, they were transferred to the incubation tank.

Figure A. Small scale *Artemia* cyst hatching system and B. Microscopic observation of hatched *Artemia* nauplii

Counting and evaluating *Artemia* nauplii

To assess the hatching results and to feed the larval rearing tanks at the established densities you have to count the *Artemia* nauplii. Three methods are described below, first for high nauplii densities, such as after harvesting and in a cold storage tank, second for counting the nauplii when they are in the incubation tank and the third for low nauplii densities, similar to those which can be found in fish tanks.

Counting high density nauplii samples

- Take a 10-ml sample of the population to be estimated.
- Dilute the nauplii concentration by adding 90 ml of sea water to obtain a total sample volume of 100 ml.
- Take three sub-samples with a 1-ml pipette, avoiding sucking air bubbles.
- Transfer each sub-sample to a Petri dish.
- Add a few drops of the fixative staining solution Lugol to each of

the Petri dishes and wait until all nauplii are immobile and deeply stained.

- Add water so as to distribute the nauplii over the whole surface of the three Petri dishes.
- Put each Petri dish on a grid and count the nauplii present in each 1 ml sub-sample.
- Calculate the average number of nauplii per ml by dividing the sum of the three counts by three.
- The total nauplii density per litre is given by the average number of nauplii per ml multiplied by 10.
- To give the number per undiluted ml and finally by 1 000 to get the final density per liter.

Counting nauplii samples from the incubation tank

- Take with a test tube a 50-ml sample of the population to be estimated.
- Take three sub-samples with an automatic 0.1-ml pipette.
- Transfer each sub-sample to a 3 cm-wide Petri dish, whose bottom has been subdivided in a 5mm-grid.
- Add a few drops of the fixative staining solution Lugol to each of the Petri dishes and wait until all nauplii are immobile and deeply stained.
- Add water so as to evenly distribute the nauplii over the whole surface of the three Petri dishes.
- Count the nauplii present in each 0.1 ml sample.
- Calculate the average number of nauplii per ml by dividing the sum of the three counts by three.
- The total nauplii density per litre is given by the average number of nauplii per ml multiplied by 10 to give the number per ml and finally by 1000 to get the final density per liter.

Counting low densities nauplii samples

- Take three 50 or 100 ml samples, using a graduated cylinder which has been cut at the 50 or 100 ml mark.
- Transfer each sample to a large Petri dish.
- Add several drops of Lugol to each of them and wait until all nauplii are immobile and deeply stained.
- Put each Petri dish on a grid and count the nauplii present in each sample.
- Calculate the average number of nauplii present in each sample.
- Calculate the nauplii density per litre by multiplying this count either by 20 (50 ml sample) or by 10 (100 ml sample).

The two main criteria to evaluate hatching results are:

- Hatching rate: the number of nauplii hatched per 100 cysts; good batches have a hatching rate around 90-95%.
- Hatching efficiency: the number of nauplii produced per gram of cysts; top quality cysts yield about 300 000 nauplii/g.

Artemia enrichment

- Prepare culture vessel for the enrichment process using fresh seawater.
- Salinity 35 - 45 (ppt); temperature: 27 -28 C and vigorous aeration.
- Harvest the newly hatched nauplii.
- Gently rinse with fresh water with suitable filter.
- Transfer to a clean culture vessel.
- Stock at the rate of 8,000 -10,000 newly hatched brine shrimp per liter
- Do not feed nauplii at this time - they are absorbing their attached yolk sac.

- Approximately 8 to 10 hours from time of transfer to clean culture vessel, the nauplii will have molted into the Instar II feeding stage.
- Add **SELCO 0.2 g per 1,00,000** Artemia nauplii per liter.
- Ensure that the aeration is vigorously mixing the water column.
- After approximately 12 hours, the intestinal tract of the nauplii should be fully enriched with **SELCO**.
- Harvest enriched nauplii and feed immediately maintaining a nauplii concentration of 2-3 nos. per ml.
- Unfed enriched nauplii can be stored in the refrigerator for later feedings .

Enrichment using Instar-II-nauplii :

Add **0.6g (in a minimum of 2 rations (0 hrs – 12 hrs)** of A1 DHA SELCO per liter of seawater containing up to 3,00,000 Artemia nauplii, in the enrichment tank. Enrich for a period of 24 hours while maintaining min, 4 ppm DO and p H of 7.5-8.5.

Optimal enrichment conditions

- For water quality and container design, see previous section; initial nauplii density: between 150,000 and 300,000 nauplii/l.
- Vigorous water agitation to keep the nauplii in suspension and pure oxygen to keep dissolved oxygen above 4 ppm throughout the enrichment period: use one open PVC pipe for air and a micro-bubbles diffuser for oxygen.
- Lighting: not required.

Prepare the enrichment meal as specified by the producer, and make sure to prepare a new enrichment emulsion for each meal. At the end of the enrichment time harvest the metanauplii as usual, rinsing them thoroughly with seawater until no oily emulsion is noticed in the outflowing water.

Marine Fin fish Diseases and health management

Introduction

Sustainable aquaculture production can only occur when fish are healthy and free from disease. Fish disease management is a combination of preventing the onset of disease and measures to reduce losses from disease when it occurs. Fish cultured in floating cages become particularly susceptible to disease when various environmental parameters such as temperature, salinity, dissolved oxygen and suspended particles fluctuate suddenly or widely, or following rough , although often unavoidable handling operation. Once conditions suitable for pathological changes develop, progress to disease in the warm water environment is rapid. Early detection of behavioural changes and clinical signs in the cultured animals are critical for proper diagnosis of the disease.

Disease rarely results from simple contact between the fish and a potential pathogen. Environmental problems, such as poor water quality, or other stressors often contribute to the outbreak of disease.

What is Fish Health Management?

Fish health management is a term used in aquaculture to describe management practices which are designed to prevent fish disease. Once fish get sick it can be difficult to salvage them. Successful fish health management begins with prevention of disease rather than treatment. Prevention of fish disease is accomplished through good water quality management, nutrition, and sanitation. Without this foundation it is impossible to prevent outbreaks of opportunistic diseases. The fish is constantly bathed in potential pathogens, including bacteria, fungi, and parasites. Even use of sterilization technology (i.e., ultraviolet sterilizers, ozonation) does not eliminate all potential pathogens from the environment. Suboptimal water quality, poor nutrition, or immune system suppression generally associated with stressful conditions allow

these potential pathogens to cause disease.

Predisposing factors

Fish stocks living under stressful conditions become less able to defend against a pathogen and hence will become sick more readily. Fish that are well cared for generally do not become sick even in the presence of a pathogen. The most common error in fish husbandry is overstocking. This leads to problems such as:

- Fish to fish aggression
- Increased fish and feed wastes
- Ease of disease spread,
- Increased concentration of pathogens
- Resultant poor water quality

High fish density, stress, and ease of transmission increase susceptibility of the fish population to diseases and parasites. In marine aquaculture, diseases present in wild fish can infect cultured fish and spread rapidly through the population

Types of Fish Diseases

There are two broad categories of disease that affect fish, infectious and non-infectious diseases. Infectious diseases are caused by pathogenic organisms present in the environment or carried by other fish. In contrast, non-infectious diseases are caused by environmental problems, nutritional deficiencies, or genetic anomalies; they are not contagious and usually cannot be cured by medications.

- **Infectious diseases**. Infectious diseases are broadly categorized as parasitic, bacterial, viral, or fungal diseases.
- **Vibriosis** is a bacterial disease causing significant losses of fish in marine fish farms. Cobia, Grouper, seabream, snapper and pompano species are affected. Vibriosis results in severe skin, muscle, fin, eye and internal organ damage of fish. Diagnosis of the disease requires bacteriological culture of kidney, spleen, skin or eye lesions.

Common Diseases of Cobia (*Rachycentron canadum*)

S.No	Bacterial disease	Causative organism
1	Pasteurellosis	*Photobacterium damsella* sub sp *pisicida*
2	Streptococcosis	*S. iniae*
3	Vibriosis	*V.anguillarum*
4	Bacterial enteritis	*V.alginolyticus*
5	Mycobacterium infection	MY. Sp 2nd *Aeromonas hydrophila*
6.	Viral disease Lymphocystis	Irido virus

Common Diseases of Pompano *(Trachinotus blochii)*

S.No	Disease	Causative agent
1	White spot disease	Ciliate protozoan,*Cryptocaryon irritans*
2	Cardiac myxosporidiosis	Myxosporidian protozoan,*Henneguya*sp
3	Monogenetic trematode infestation	*Bicotylophora trachinoti*- gills *Benedenia* sp- body
4	Fatty degeneration	Dietary deficiency- protein
5	Parasitic dermatitis (infestation)	Sea lice (*Calligus elongatus*)

- **Non-infectious diseases**: Non-infectious diseases can be broadly categorized as environmental, nutritional, or genetic.

A hygienic fish culture environment is essential to the health and productivity of farming operations. The reasons for this include:

- Disease risks are increased in poor and polluted environments.
- Quality of the product depends on clean and healthy environments.

The culture environment incorporates the following components

- Physical farm infrastructure e.g. fish cages, floats, nets, and utensils.

- Water quality e.g. dissolved oxygen and microbial contamination.
- Seabed sediments e.g. solid wastes measured as carbon, nitrogen and phosphorus.
- Introduced chemicals e.g. antibiotics, metals and pesticides.

Farm hygiene is vital to maintaining fish health. It involves routine activities carried out by the farmer to ensure the following:

- Removal of biofouling from net/ pens.
- Cleaning of utensils and equipment used to handle or feed fish.
- Water quality testing and correction of poor water quality includes the following:
- Measure dissolved oxygen in water
- Chemistry values e.g. salinity, temperature, pH, ammonia, nitrite and nitrates.
- Measure bacterial counts e.g. *Vibrio* spp. counts of the water
- Aeration to maintain dissolved oxygen
- Cleaning of the farm seabed and fallowing or rotation of sites
- Minimising organic pollution from fish wastes and feed wastes

Preventive measures

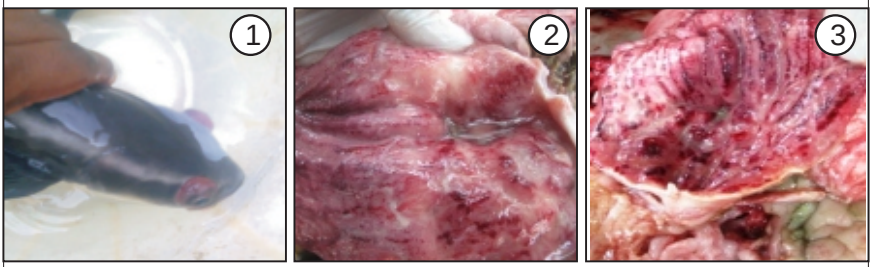
- Preventing the introduction of pathogens
- Maintenance of good water quality
- Avoidance or reduction of environmental stressors
- Adequate nutrition
- Isolation of cultured animals from feral stocks
- Immunization

Three steps to solve a disease problem

- Determining that a problem exists.
- Identifying the cause of the disease or source of the distress
- Successfully curing the fish and eliminating the disease or cause of distress.

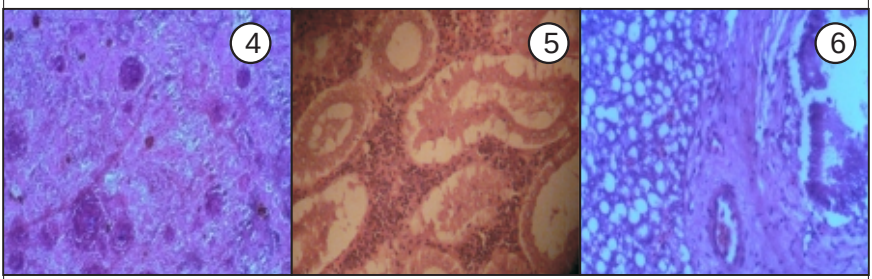
Pictures:

1) Fingerlings affected with Vibriosis



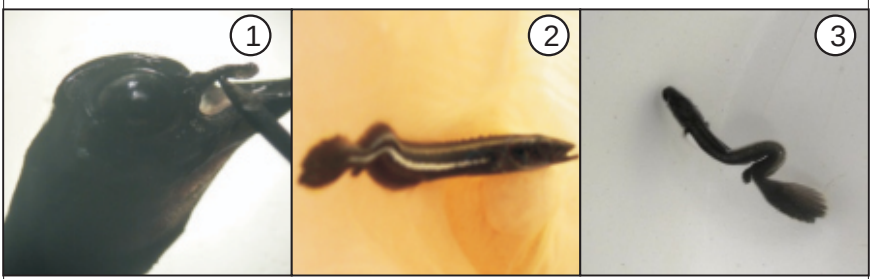
1, Eye: ***Bilateral exopthalmus*** **2-3, Stomach:** ***Haemorrhagic gastritis***

2) Histopathology :



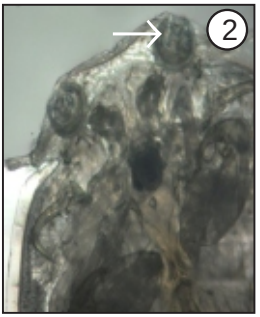
4. Spleen:Liquefactive necrosis-H&E ***5. Kidney : Acute tubular necrosis H&E*** ***6. Liver : Fatty degeneration -H&E***

3) Genetic anomalies

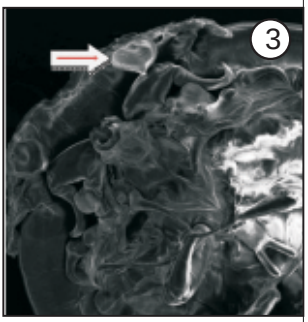


1. Undeveloped upper maxilla 2. Scoliosis- vertebral anomalies

4) Pompano affected with sea lice (*Calligus elongatus*) infestation

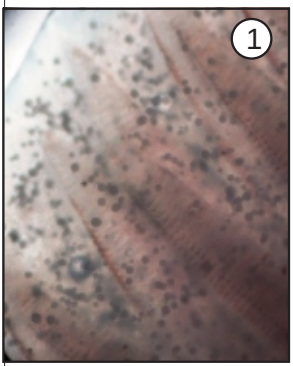


1&2. Microscopic image of*Caligus elongatus*

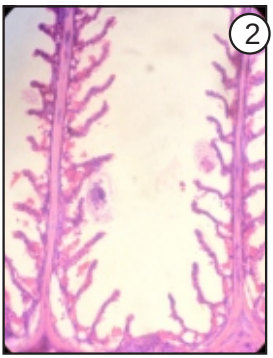


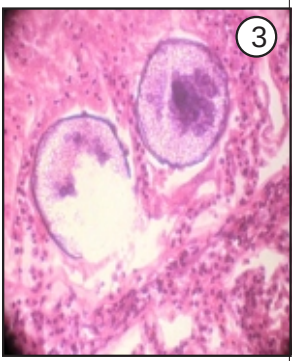
3) SEM view presence of lunules (arrow)

5) Pompano parasitic infestation due to *Amyloodinium ocellatum*

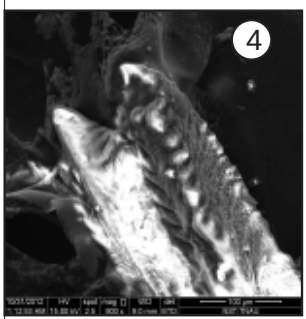


1. *A. ocellatum:* Gill- Adult feeding stage-trophont

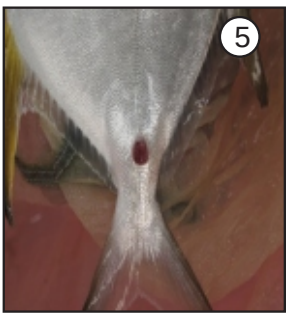




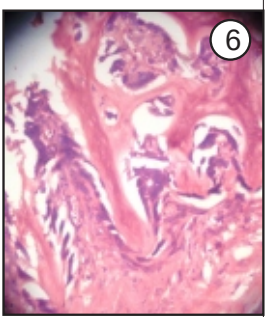
2-3. Gill: Hypertrophy of the secondary lamellae H&E



4. SEM Gill: Hypertrophy



5. Pompano : Tail tumour



6. Tail tumour :Papillary cyst adenoma H&E

NOTES