Training manual on

Hatchery Production

and

Farming of Marine Finfishes

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Training Manual on

Hatchery production and farming of marine finfishes

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Live Feeds: Culture of Microalgae, Rotifer and Artemia

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Importance of live feeds in aquaculture

Live feed refers to small, microscopic living organisms which are used to feed fish larvae especially during the early larval stages. Live food organisms include all plants (phytoplankton) and animal (zooplankton) lives grazed upon by economically important fishes. Phytoplankton are generally eaten by zooplankton and forms the basis of the food chain. Live foods can swim in water column and are constantly available to fish and shellfish larvae and are likely to stimulate larval feeding response. Most of the fish and shellfish larvae in nature feed on small phytoplanktonic and zooplanktonic organisms. The success in the hatchery production of fish fingerlings for stocking in the grow-out production system is largely dependent on the availability of suitable live food for feeding fish larvae, fry and fingerlings. Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids and hence are commonly known as "living capsules of nutrition".

Upon hatching of fish eggs, larvae obtain their nutrition from yolk sac for the first few days. Generally, there are two types of fish larvae: precocial and altricial. Precocial larvae are those that when yolk sac is exhausted, appear as mini adult exhibiting fully developed fins and mature digestive system. Such fish can ingest and digest formulated diet as first food. But altricial larvae (most of marine fish larvae) having less yolk reserves remain in a relatively undeveloped state with small mouth gape when yolk sac is exhausted. They also possess an undeveloped digestive system lacking a stomach. These types of larvae cannot digest formulated diet and has to be provided with live feeds appropriate to their mouth gape. Live feeds are able to swim in water column and thus constantly available to the larvae. In addition the movement of live feed in the water is likely to stimulate larval feeding responses since evolutionary history has probably adapted them to attack moving prey in nature.

Selection of live feed

When selecting food to be fed to the larvae, the following points should be considered:

1. The food must be perceived by the larvae. 2. The size of food must be such that it can be accommodated by the mouth of the larvae. 3. The feed should have high nutritional quality especially Highly Unsaturated Fatty Acids (HUFA) essential to the growth and survival of the larvae. 4. The feed can be digested by the larvae. 5. Food organisms must be hardy. 6. They must be able to reproduce rapidly and can mass produced under controlled condition

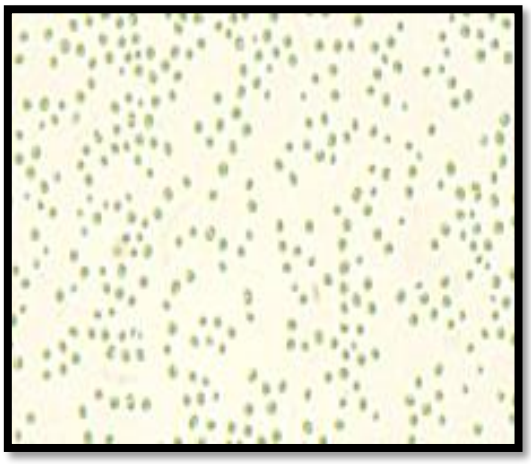
MARINE MICROALGAE

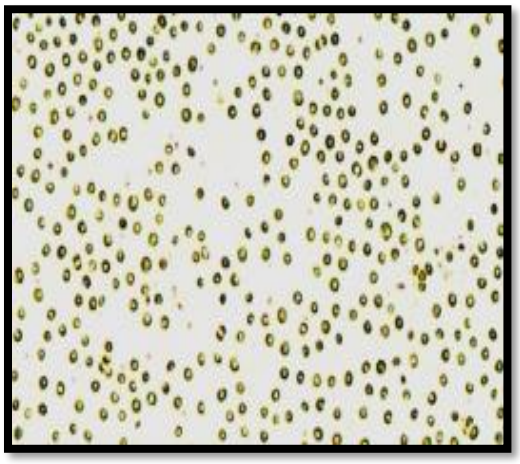
Microalgae are the primary food source of all marine food chains and provide energy for all successive trophic levels in the marine ecosystem. They are generally free living, pelagic and in the nano- plankton range (2-20µm). According to the nature of photosynthetic pigment, algae are further classified into three divisions such as Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae). Their role is critical in a successful mariculture hatchery management. Microalgae remain an indispensable hatchery food for many fish species, despite some on-going progress in the development of formulated larval feeds. They are utilized during all growth stages of filter feeders (e.g. oysters, scallops, clams and mussels), for larval/early juvenile stages of abalone, crustaceans and some fish species, and as well as used for the production of zooplanktons such as rotifers, copepods etc. in hatchery, which in turn are used for the carnivorous larvae of many of the fish species.

Microalgae in the larval rearing tank is considered

- To stabilize the water quality in static rearing systems (remove metabolic byproducts, produce oxygen)
- To be a direct food source for the larvae
- To stimulate the non-specific immune system in the larvae
- To be an indirect source of nutrients for fish larvae through the live feed
- To increase feeding incidence by providing background
- To control microbes in tank water and/or larval gut

The prime requirements of aquaculture practice are the production of appropriate nutritionally balanced, non- polluting, economically viable and readily acceptable micro algae which will ensure maximum survival and optimum growth of cultivable organisms. Nearly 16 genera of microalgae are commonly employed for aquaculture purposes. Species mainly used ones are belongs to the genus *Isochrysis, Nanochloropsis, Pavlova, Chatoceros, Dunaliela, Tetraselmis* and *Chlorella.*



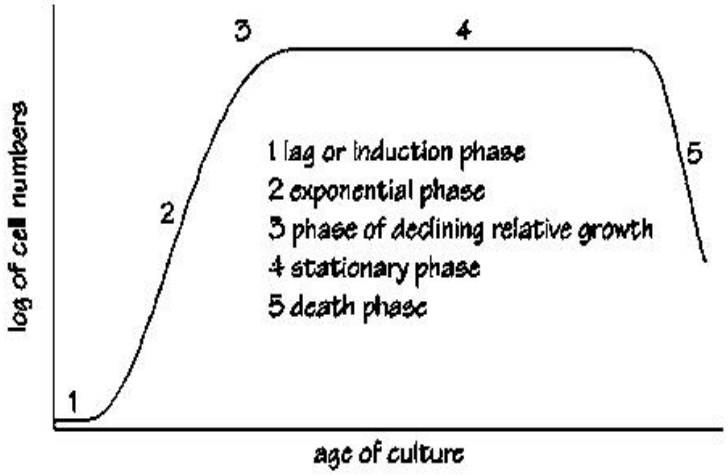


Nanochloropsis ***Isochrysis***

Growth dynamics

The growth of an axenic culture of micro-algae is characterized by five phases

1. Lag or induction phase: in which there is no increase in cell numbers
2. Exponential phase or growing phase: in which cell multiplication is rapid
3. Declining phase: in which the growth and multiplication of cells will be arrested and slowly the cells shows symptoms of decline
4. Stationary phase: in which the culture will be stationary without any further cell division for a few days. In the stationary phase if the cells gets a new environment, they must start further growth and reproduction
5. Death phase: in which the cells will lose its viability and start dying. At this stage culture will become useless either for re –culturing or for feeding



Growth phases of micro algae

The growth cycle of microalgae would be helpful for successful operation of micro algae culture in a hatchery. For feeding, the culture should be given during its exponential phase only otherwise metabolites of the micro algae such as oil will be more and that will affect seriously on the rearing organisms.

Algae culture media

Algae media refers to the solution or culture in which algae grow. All the media have several components in common: sources of phosphorus, vitamins and trace metals. However the specific types of these nutrients, their concentrations and ratios vary between the media.

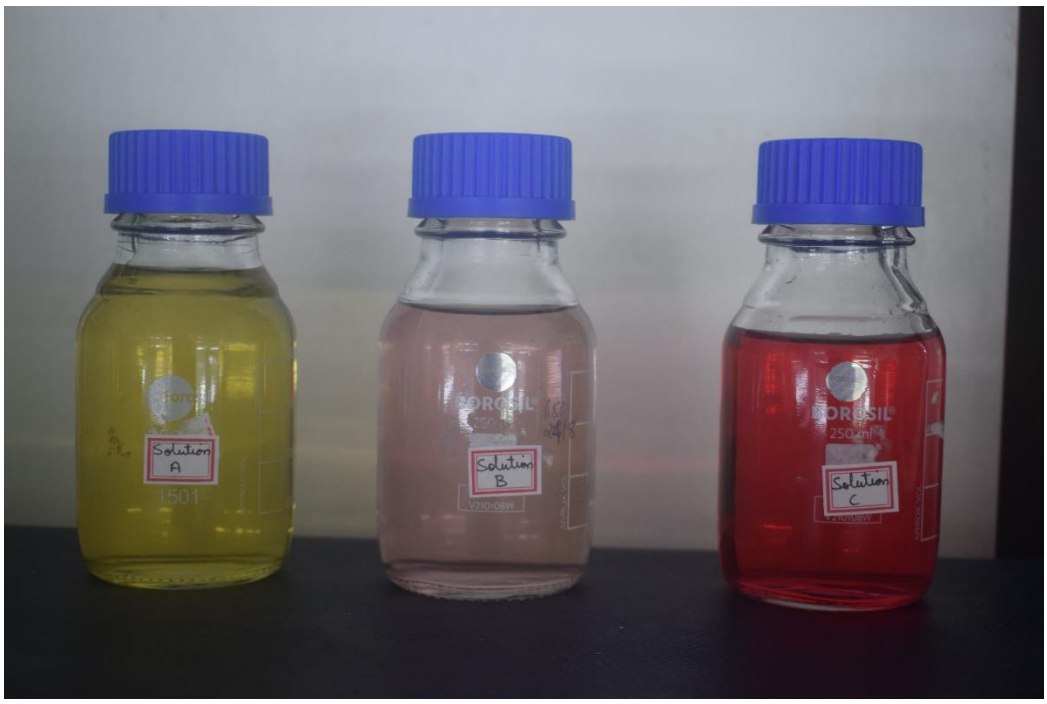
For the successful production of microalgae various culture media have been used depending on the organisms to be cultured. The two commonly used media – F/2 media and Conway or Walne's media. Walne's or Conway media contains all the ingredients and widely used in the laboratory maintenance. It is economic to use commercial fertilizers for the mass production of microalgae in outdoor mass production systems.

Composition of Walne's media

For making 1 litre of Solution A (Nutrient solution) in distilled water		
	Potassium nitrate	100 gm
	Sodium orthophosphate	20 gm
	EDTA disodium salt	45 gm
	Boric acid	33.4 gm
	Ferric chloride	1.3 gm
	Manganese chloride	0.36 gm
For making 1 litre of Solution B (Nutrient solution) in distilled water		
	Zinc chloride	4.2 gm
	Cobalt chloride	4.0 gm
	Copper sulphate	4.0 gm
	Ammonium molybdata	1.8 gm
For making 1litre of Solution C (Vitamin solution) in distilled water		
	Vitamin B1 (Thiamin)	2 g
	Vitamin B12 (Cyanocobalamine)	100 mg
For making 1litre of Solution D (Vitamin solution) in distilled water		
	Sodium silicate	40ml

These primary stock solutions have to be prepared in separate reagent bottles and should be autoclaved except the vitamin solution. From this primary stocks, the working solution is 1 ml of solution A, 0.5 ml of solution B, 0.1 ml of

solution C and 1ml of solution D (only for diatoms) is added in autoclaved reagent bottle and made up to 1 litre using filtered and sterilized seawater.



Primary stocks of Walne's media

Sterilization of seawater

For the sustained production of any algae production sterilized seawater is required. The sterilization can be done by many ways like filtration, autoclaving, pasteurization, UV irradiation, chlorination or ozonization. Care must be taken to eliminate chances of contamination from microorganisms like ciliates.

Optimal conditions for culture of microalgae

The factors which affect the growth of micro algae are light intensity, temperature control, aeration, nutrients inoculums and desired salinity. These factors are critical for maintaining a stock room in a successful way.

Parameters	Range	Optimum
Temperature (°C)	16-27	18-24
Salinity (ppm)	12-40	20-24
Light intensity (Lux)	000-10000	2500-5000
Photoperiod Hrs (L:D)	16:8	24:0
pH	7-9	8.0-8.5

Stock culture development and maintenance

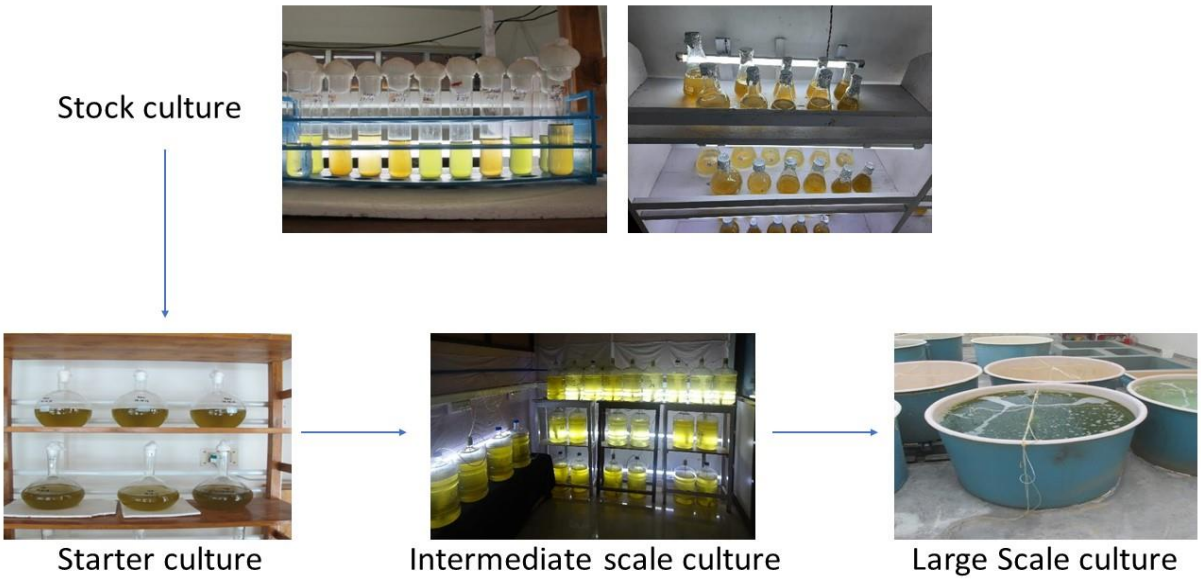
Stock cultures or master cultures are considered as the basic foundation of culture. To reduce risks of contamination, two series of stocks are often maintained, one which is usually used for the starter cultures for the production

system and the other which is only subjected to the handling necessary for stock maintenance. Stock cultures are maintained in test tubes (50 ml) or conical flaks (100-250 ml) at a light intensity of about 1000 lux and a temperature of 19 to 22 °C.

Procedure for preparation of stock culture

- Remove the cotton plugs of autoclaved culture tubes or flaks and heat sterilize using burner flame
- Fill half the test tube or conical flask with working solution of Walne's media
- Then aseptically add 10% of pure culture of desired microalgae to the test tube or conical flask
- Mouths of tubes containing the stock should be heat sterilized before covering it with cotton plugs
- Label the flask and place it in front of the light source (500Lux) in a room temperature of 22℃
- Then from these culture tubes, new stock cultures can be prepared after 10-15 days intervals to maintain them in a vigorous and healthy state

Stock cultures usually make in a small quantity and some of these cultures keeps to meet unforeseen conditions like contamination and the remaining cultures can be use to produce culture in large volume by inoculating in bigger glasswares to produce starter cultures (upto 3-4 L), intermediate culture (up to 20 L) and large scale cultures (above 50 L).



Starter cultures (250 ml to 4 l in volume) are grown quickly for 7 to 14 days for flagellates and 3-5 days for diatoms. When ready, a small portion of the volume is used to start a new starter culture and the main portion to begin an intermediate-scale culture. Intermediate scale cultures (usually of between 4 l and 20 l in volume) may be used as food for larvae or to start a large-scale cultures after 7-14 days. Most laboratories and hatcheries requiring small volumes of algae for food use plastic buckets or clear plastic carboys of up to 25 l volume. These are generally operated as batch culture systems. In this method the entire culture is harvested

when the cell density reaches the desired level. Then the culture tank is filled with enriched water and the required inoculum is added. When the cell density reaches the desired level the entire culture is harvested. Batch culture method is the most reliable method, but it is labour intensive.

Transfer protocol

The following procedures should always be used when preparing media or transferring cultures:

- work in clean place or preferably in a laminar flow cabinet (cabinet must be turned on at least 30 minutes before transfer; if equipped with UV lamps, leave on overnight prior to use).
- clean working surface with 70% alcohol (ethanol/isopropanol) prior to and after use.
- clean hands with disinfecting soap and rinse with 70% alcohol prior to all operations.
- when not using a laminar flow cabinet (and to be safe even when using a cabinet), sterilise (flame) the neck of vessel of origin before and after transfer (not possible with some plastic vessels, which must, therefore, be opened in a laminar flow cabinet).
- pipettes must be clean and sterile; use autoclavable tips for repeating pipettes, pre-wrapped sterile single use plastic/glass pipettes, or if using non-sterile glass pipettes (with cotton plugs), sterilise in the flame before use

Outdoor culture of microalgae

Large scale outdoor culture of microalgae required for hatcheries can be carried out economically by enriching with agricultural fertilizers like ammonium sulphate, urea and super phosphate in a ratio of 10:1:1.

Agricultural fertilizer/1 tonne	**Weight**
Ammonium phosphate	100 g
Urea	10 g
Triple super phosphate	10 g

Harvesting the culture

The culture should be harvested during the exponential stage of growth after determining the cell density. The culture in the declining or death phase, should not be used for any feeding because the metabolites will be more and cells may not be in good condition.

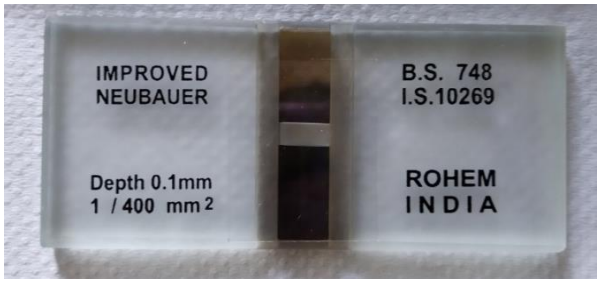
Cell density determination of culture

For determining cell density, haemocytometer is used. Haemocytometers are thick glass slides with two chambers on the upper surface, each measuring 1.0 x 1.0 mm. A special cover slip is placed over these two chambers giving a depth of 0.1 mm making the total volume of each chamber 0.1 mm^3. The base of each chamber is marked with a grid to aid in counting cells within the area. Prior to counting motile algal species, 1 or 2 drops of 4% formalin should be added to a 10

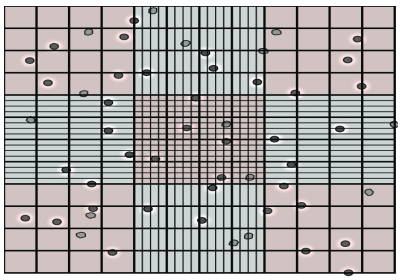
to 20 ml sample of the culture to be counted. With the cover slip in position, one or two drops of the algal sample are introduced by means of a pasteur pipette to fill both chambers. Cell density is estimated as follows. The central grid of each chamber is sub-divided into 25 squares, each measuring 0.2 x 0.2 mm. The numbers of cells in 10 randomly chosen 0.2 x 0.2 mm squares are counted and the average or mean is calculated. This gives the mean number of algal cells per 0.2 mm x 0.2 mm x 0.1 mm, or 0.004 mm^3. During counting, cells touching the outer most line of the left hand and top square edges are counted. Cells touching the bottom or right-hand edge are not counted. After counting the cells, the haemocytometer and cover slip should be rinsed with distilled water.

Calculation of cell density

Cell density (cells/ml) = average number of cells× 250x1000



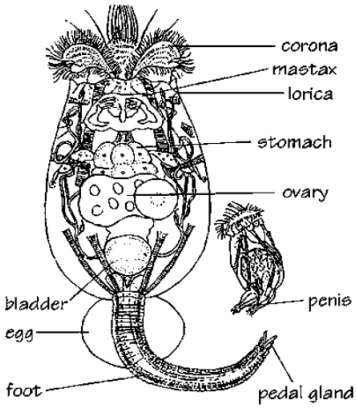
Haemocytometer



Haemocytometer under microscope

ROTIFERS

Rotifers are very small organisms mostly ranging from 0.1 to 0.5 mm belongs to the Phylum Rotifera. Rotifers were found to be suitable as first feed for marine fish larvae in Japan in the late sixties and early seventies. Rotifers became ubiquitous in all mass rearing trials after their successful use in the mass rearing of the red seabream *(Pagrus major)* in Japan. The introduction of rotifers marked the first regular successes in the mass larval rearing of several marine species of economic value such as grey mullet *(Mugil cephalus),* sole *(Solea solea)*, gilthead seabream *(Sparus aurata)*, sea bass *(Dicentrarchus labrax)*, turbot *(Scophthalmus maximus)* and flounder *(Paralichthys olivaceus)*, milkfish *(Chanos chanos)* etc.. during seventies and eighties. They are now the first food of the initial larval stage of many fish species grown in commercial marine hatcheries.

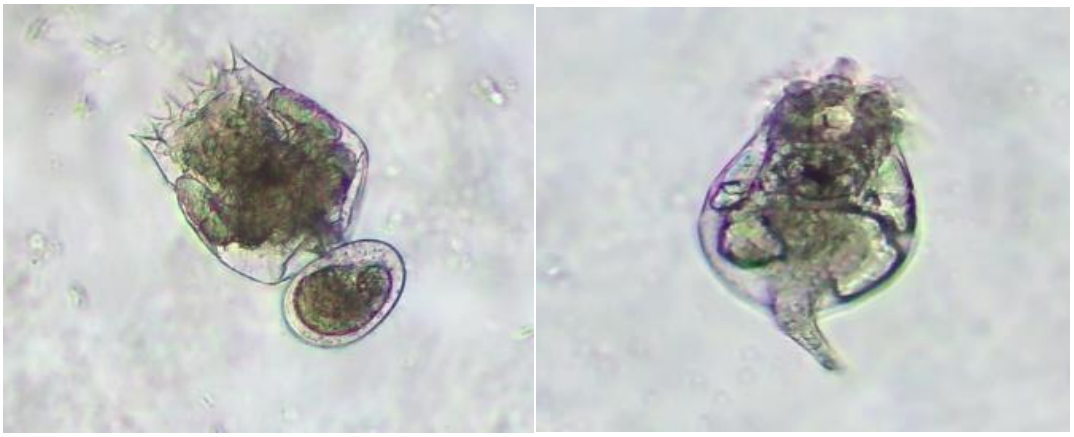


Morphology of rotifer

The success of rotifers as a culture organism are manifold, including their planktonic nature, tolerance to a wide range of environmental conditions, high reproduction rate. Moreover, their small size and slow swimming velocity make them a suitable prey for fish larvae that have just resorbed their yolk sac but cannot yet ingest the larger *Artemia* nauplii. However, the greatest potential for rotifer culture resides, however, is the possibility of rearing these animals at very high densities. Even at high densities, the animals reproduce rapidly and can thus contribute to the buildup of large quantities of live food in a very short period of time. Last, but not least, the filter-feeding nature of the rotifers facilitates the inclusion into their body tissues of specific nutrients essential for the larval predators.

Rotifers are capable of reproduction though both sexual and asexual methods. The life span of rotifers has been estimated to be between 3.4 to 4.4 days at 25°C. Generally, the larvae become adult after 0.5 to 1.5 days and females thereafter start to lay eggs approximately every four hours. It is believed that females can produce ten generations of offspring before they eventually die.

Euryhaline rotifer species, *Brachionus* are used extensively used for marine fish larval rearing in many hatcheries throughout the world. *B. plicatilis* (L type) and *B. rotundiformis* (S-type) are the two common species used for larviculture of marine fishes. The differences among the two types can be clearly distinguished by their morphological characteristics: the lorica length of the L-type ranging from 130 to 340 mm (av. 239 mm), and of the S-type ranging from 100 to 210 mm (av. 160 mm). Moreover, the lorica of the S-type shows pointed spines, while of the L-type has obtuse angled spines. Another strain. SS-type rotifers (Super small rotifers) are preferred for the first feeding of fish larvae with small mouth openings (rabbitfish, groupers, and other fish with mouth openings at start feeding of less than 100 mm). Those rotifers, however, are genetically not isolated from S-strains, but are smaller than common S-strains.



B. rotundiformis ***B. plicatilis***

Stock culture

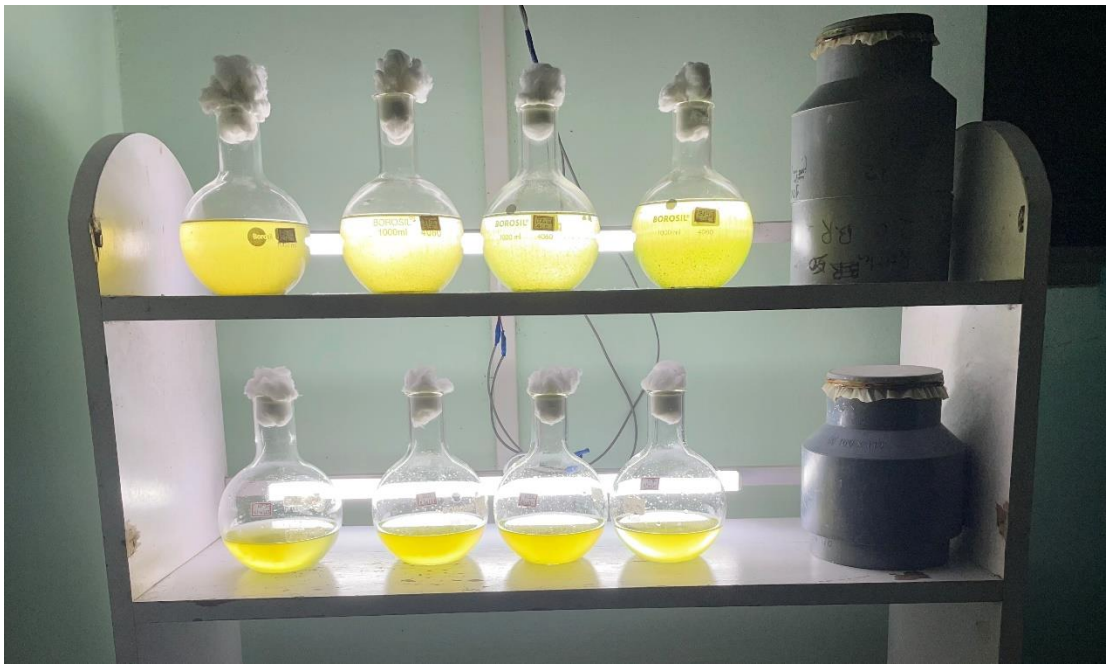
Relying only on mass cultures of rotifers for reinoculation of new tanks is too risky an approach because the threat of mass mortality. In order to minimize this risk, small stock cultures are generally kept in closed vials in an isolated room to

prevent contamination with bacteria and/or ciliates. These stock cultures which need to generate large populations of rotifers as fast as possible are generally maintained on algae.

- Sterilized seawater of 25 ppt salinity is used
- Inoculation of the tubes (50 ml) is carried out with an initial density of 2 rotifers.ml^{-1}.
- The tubes are placed at a distance of 20 cm from fluorescent tubes (light intensity of 3000 lux)
- 4 ml of microalgae is added daily
- After one week the rotifer density should have increased from 2 to 200 individuals/ml. The rotifers are rinsed, a small part is used for maintenance of the stock, and the remaining rotifers can be used for upscaling.

Upscaling of stock cultures to starter cultures

The upscaling of rotifers is carried out in static systems consisting of conical flask of 500 ml placed 2 cm from fluorescent light tubes (5000 lux). The rotifers are stocked at a density of 50 individuals.ml^{-1} and fed 400 ml freshly-harvested algae (10^6 cells.ml^{-1}). Next two days rotifers are fed approximately 50 ml of algae. Within 3 days the rotifer concentration can increase to 200 rotifers/ml.



Mass culture

Batch culture system is probably the most common method of rotifer production in marine fish hatcheries. The culture strategy consists of either the maintenance of a constant culture volume with an increasing rotifer density or the maintenance of a constant rotifer density by increasing the culture volume. Batch culture system normally follows a 4–5-day culture period. Initially a tank (0.5-1 ton) is inoculated with rotifers (≥ 200 nos./ml). The rotifers are then fed each day with microalgae (10^6 cells/ml) and the volume of the culture is also increased to keep up with rotifer growth. Many species of microalgae such as *Nannochloropsis*, *Chlorella*, *Pavlova*¸ *Isochrysis* etc, are used for feeding rotifers. Rotifer densities

reaches up to 500 nos. /ml after 4-5 days. Rotifers are harvested after the culture period using 300 µ and 50 µ mesh sieves, so that dust free rotifer will be collected on the 50 µ mesh sieves. Both filters are placed inside a container full of water to avoid damage to the rotifers due to pressure of outgoing water. A gentle air bubbling along the inner side of the filter helps to keep the filter free from clogging. A part of the harvested rotifers are used as the innoculum for the next culture.

Rotifers are also cultured using a combination of microalgae and baker's yeast and promotes better growth of rotifer cultures. The tanks are half filled with algae (10^6 cells/ml) and inoculated with rotifers at a density of 100 nos./ml. The first day active baker's yeast is administered two times a day at a quantity of 0.25 g/10^6 rotifers. The next day the tanks are completely filled with algae at the same algal density and 0.375 g baker's yeast per million rotifers is added twice a day. The next day the rotifers are harvested and new tanks are inoculated (i.e. two-day batch culture system)

Ideal water quality parameters for rotifer culture

Salinity	10 - 35ppt
Temperature	20- 28°C
Dissolved Oxygen	above 3 ppm
pH	7.5-8.5
Ammonia	below 1 ppm

Rotifers are often cultured at temperatures and salinities different from the larval rearing tanks. So they have to be acclimated for at least 6 hours to larval rearing conditions before introducing into larval rearing tanks.

Enrichment

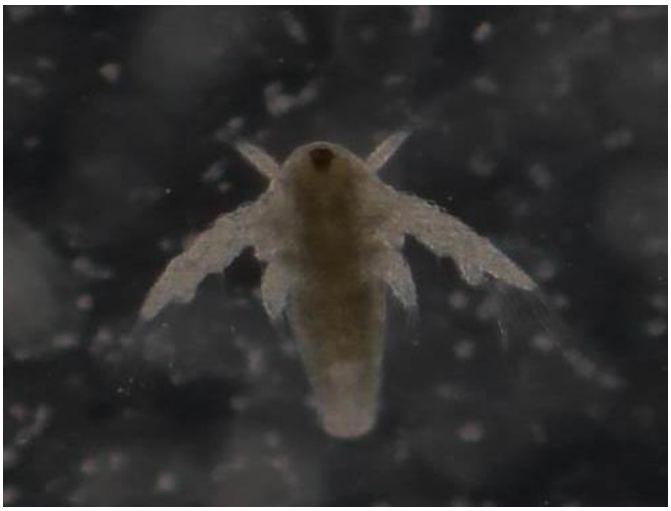
Rotifers have a limited nutritional value for fulfilling the nutritional requirement of marine finfish larvae. The high content of the essential fatty acid eicosapentaenoic acid (EPA 20:5n-3) and docosahexaenoic acid (DHA 22:6n-3) in some microalgae (*e.g.* 20:5n-3 in *Nannochloropsis occulata* and 22:6n-3 in *Isochrysis galbana*) have made them excellent live food diets for boosting the fatty acid content of the rotifers. Rotifers are enriched by stocking the harvested rotifers @ 500 nos./ml in microalgae cultures (10^6 cells/ml) for 4-6 hrs. It can also be enriched with specially formulated artificial diets like Selco products or using homemade or commercial oil emulsions.

ARTEMIA

Among the live diets used in the larviculture of fish and shellfish, nauplii of the brine shrimp *Artemia* constitute the most widely used food item. It is also called as brine shrimp or sea monkey. The total length is about 8-10mm for adult males and 10-12mm for females, the width including legs is about 4mm for both sexes. The widely used species of *Artemia* is *A. salina*. The females can produce eggs either as a result of mating or via parthenogenesis. The thin shelled eggs hatches immediately, and thick shelled eggs can remain in dormant state and forms cysts. These cysts are metabolically inactive, do not further develop as long as they are kept dry and can be stored up to 2 years. Upon immersion in seawater, the biconcave-shaped cysts hydrate, become spherical, and within the shell the embryo

resumes its interrupted metabolism. In less than 24 hrs, the outer membrane of the cyst bursts and the embryo appears surrounded by the hatching membrane. While the embryo hangs underneath the empty shell, the development of the nauplii is completed and within a short period of time the hatching membrane is ruptured and the free-swimming nauplii comes out. This freshly hatched nauplius I stage (Instar I) (length of 400-500µ) is popularly used for feeding the larvae.

Each gram of *Artemia* cyst contains 200000 to 300000 eggs and atleast 50% will hatch within 20-24hrs on proper hydration. To estimate the amount of *Artemia* required one must consider both the volume of the tank and the expected number of *Artemia* the larvae will consume. Based on the stage or the age of the larvae, estimate a daily *Artemia* requirement per ml. *Artemia* nauplii are usually maintained in the larval culture tank at densities of 0.5 to 2 per ml for most species of finfishes The requirement of *Artemia* nauplii should be calculated based on the volume of larval rearing tank and the species under culture. Also, *Artemia* nauplii left over in the tank should be determined daily by taking water samples from the larval rearing tank to back calculate the requirement of nauplii/l and requirement of cysts in g for producing the required amount of nauplii.



Weight of *Artemia* cyst required =

Total volume of all rearing tanks (in ml) X No. of *Artemia* required per ml

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Percentage hatch rate X No. of cysts per gram

Artemia nauplii when required in large quantities, it is essential to disinfect the cysts before hydration to reduce the bacterial load to increase the quality and quantity of hatching.

Procedure for hatching *Artemia* cysts in hatchery

Artemia cysts are hatched into nauplii following the standard procedure involving the following steps

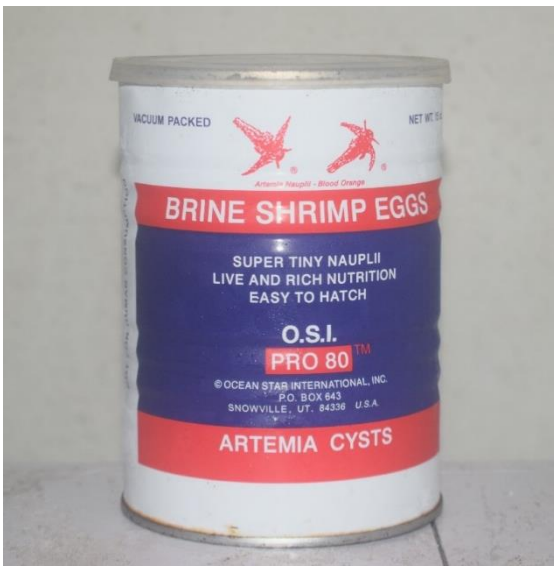
1. Hydration of cyst

Artemia cysts (<100 g/l) are put into container containing low salinity water or freshwater at 25°C for 1 hour with aeration. After an hour, hydrated cysts are filtered through 100- 125 µm sieve. Hydration steps ensures the spherical shape of the cyst, which allows for easy removal of cysts.

2. Decapsulation

Decapsulation procedure ensures disinfection of cysts, complete removal of shell from the hydrated cysts, minimize illumination requirements for hatching, reduces hatching time and increases nutritional value of nauplii.

a. Using Sodium hypochlorite: Hydrated cysts are kept in a beaker filled with 5% sodium hypochlorite solution @ 15 ml for every 1 g cyst. In order to prevent the temperature rising above 300 °C, the beaker containing the hydrated cyst and sodium hypochlorite are kept in a trough containing cool water or ice. In about 10 minutes, chorion of hydrated cysts gets dissolved and is then filtered through a 100 µm sieve.



b. Using Calcium hypochlorite: The scoop net containing hydrated cysts are kept in a bucket filled with water containing calcium hypochlorite having 200-250 ppm of chlorine. In about 10 minutes, chorion of hydrated cysts gets dissolved and is then filtered through a 100 µm sieve.

Note: The decapsulated cysts should be thoroughly washed in tap water or seawater for about 10 minutes until no chlorine smell can be detected. To ensure complete removal of chlorine, the cysts are dipped in 0.1% sodium thiosulphate solution or 0.1N hydrochloric acid.

3. Hatching/ Incubation of decapsulated cyst

Decapsulated *Artemia* cysts are hatched in a cylindroconical FRP tanks/jars having transparent bottom with continuously aerated from the bottom with air-lines. Decapsulated cysts are stocked @ 5 g/l of seawater in smaller volume tanks (< 20 L) to 2 g/l of seawater in tanks with large volumes. The optimum water quality parameters required for hatching of cysts is 27-30°C temperature, 8-8.5 pH, 25-35 ppt salinity, 1000- 2000 lux light and saturated dissolved oxygen concentration

4. Harvesting of nauplii.

The decapsulated cysts hatch out after a period of 16-24 hrs. Harvesting is done by taking advantage of phototactic movement of *Artemia* nauplii. The top of the cylinder is closed with a lid and illumination is provided at the bottom transparent part. After 5-10 minutes, the nauplii gets accumulated at the bottom. The outlet of the tank at or near bottom is opened and water is sieved through 100-125 µm net to harvest the hatched out nauplii. Then harvesting net should be submerged in water all the time so as to prevent physical damage of the nauplii. They are then rinsed thoroughly with water to remove possible contaminants and hatching metabolites.

***Artemia* enrichment**

Since *Artemia* have poor content of essential fatty acids, eicosapentaenoic acid (EPA: 20:5n-3) and even more importantly docosahexaenoic acid (DHA: 22:6n-3),

modification of the nutritional value of *Artemia* for aquaculture purposes has been done for years. Taking advantage of the primitive feeding characteristics of *Artemia* nauplii, it is possible to manipulate the nutritional value of HUFA-deficient *Artemia*. Since second instar stage (about 8 h following hatching) are non-selective particle feeders, simple methods have been developed to incorporate different kinds of products into the *Artemia* prior to feeding to predator larvae. This method of bioencapsulation, also called *Artemia* enrichment or boosting, is widely applied in marine fish and crustacean hatcheries for enhancing the nutritional value of *Artemia* with essential fatty acids.

Artemia are non-selective filter feeders and therefore will ingest a wide range of foods. The main criteria for food selection are particle size, digestibility, and nutrient levels. The best feeds for *Artemia* are live microalgae and the combinations of live phytoplanktons fed to *Artemia* cultures have demonstrated superior enrichment characteristics (i.e., increased HUFAs). In addition to live algae, *Artemia* cultures can be enriched by feeding a wide variety of processed foods, including yeasts, fish meal, soybean powder, egg yolk, and micronized rice bran. *Artemia* are enriched with commercially available enrichment medium (DC DHA Selco, algamag, ORI-N3 Skretting). The enriched, *Artemia* are rinsed, concentrated, counted, and then fed to larvae.

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Maintenance and culture of copepods

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Copepods are the most common planktonic crustaceans that occur in almost all kinds of water bodies on the earth's surface. There are more than 210 families, 2400 genera and 24,000 species identified in this group. Planktonic copepods are considered to be the most abundant metazoans on earth. From the Lower Cretaceous period onwards, these groups were diversified and adapted to almost all kinds of aquatic habitats and successfully colonized everywhere. In the marine environment, copepods are present in all types of water bodies from pelagic to deep sea and from sea shore to deep hydrothermal vents. Some of them are adapted to live inside the body cavity of many animals. These form the important secondary producers/primary consumers and ultimately contribute significantly to the food chain in large ecosystems. Almost all types of marine organisms, directly or indirectly depend on these small organisms for their food. Copepods form important food for many marine fishes and invertebrates. Certain fishes and fish larvae were evolutionarily adapted for feeding on copepods. Copepods are nutritionally superior than almost all live feeds. Many fishes, especially those with weak fish larvae, totally depend on copepod nauplii for their survival at least for the initial few days. Due to their smaller naupliar stages and nutritional superiority, copepod cultures became an integral component in marine finfish hatcheries.

Copepods are much superior in nutrition than most of the popular and common live feeds in the hatchery. Feeding marine fish larvae with copepods increases their survival, growth rates, reduces deformities and enhances pigmentation and stress tolerance more than almost all other popular live feeds.

Among the planktonic copepods, three major groups are very important in terms of live feeds- calanoids, cyclopoids, and harpacticoids. All these three groups are being utilized as live feeds in the hatcheries. There is always a limitation in the high-density cultivation of copepods. Rotifers can be cultured to a high density of 2000/ml. But in the case of most of the copepod cultures, the density rarely exceeds 2-3/ml for adults and 10/ml for nauplii. Calanoids are comparatively more difficult to culture than cyclopoids and harpacticoids in small quantities. But large-scale cultures are more easy for calanoids. Calanoids have smaller pelagic larvae and can be more easily produced on a large scale in hatcheries. Copepods that scatter their eggs are ideal for large-scale cultivation in hatcheries.

Species and culture

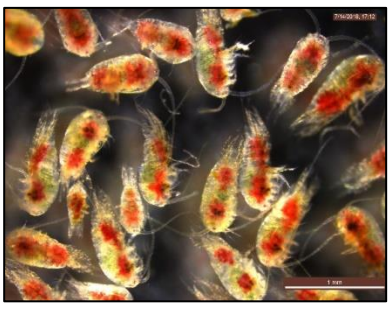
More than 60 species of copepods have been raised in laboratories but very few are popular for large scale production. Copepods can be cultured extensively, intensively, and semi-intensively. The extensive cultures are mainly in tanks, outdoor ponds, lagoons or enclosed fjords. By using sieves of appropriate mesh sizes, these cultured copepods can be made available to fish larvae. In

extensive systems, culture is done normally by producing microalgal blooms using ordinary agricultural fertilizers. Agriculture fertilizers, both organic and inorganic, were used with or without the combination of fishmeal, rice bran, wheat bran and fish feeds as inputs for nutrients. But here the main disadvantage is the unpredictable nature of production. Semi-intensive culture is generally carried out in indoor tanks with a regular supply of microalgae in combination with other inert feeds. Here regular harvest is possible and yields a mixed culture of different species of copepods. Here also the culture may not be stable for longer periods. CMFRI recommends intensive culture for large-scale production. Intensive culture is developed generally by maintaining a selected isolated pure culture of copepod species with desired qualities. Basically, there are small stock culture units, large mass culture units, and modified culture tanks fitted with structures for harvesting naupliar stages on regular basis. Specialized nauplii collection units can be attached to mass culture units also. All water quality parameters were regularly monitored and adjusted.

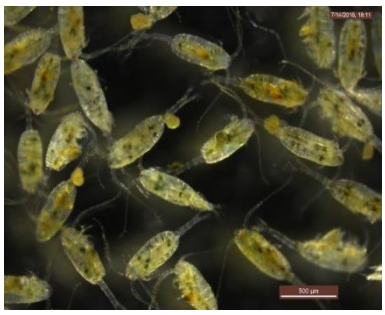
Though intensive culture may not be economical, most of the hatcheries prefer this because of the assured production of copepods and nauplii of desired size and species. This will help larvae to thrive well during critical periods of larval rearing. Once the critical period is crossed, the larvae can be fed with enriched rotifer and *Artemia*.

Copepod species developed for use as live feed

Pure stock and mass cultures of 12 species of copepods that have been identified as suitable for larval rearing are maintained at Vizhinjam Research Centre of CMFRI. Calanoid copepods (*Temora turbinata, Pseudodiaptomus serricaudatus, Acartia spinicauda, A. bilobata, A. southwelli, A. tropica, Parvocalanus crassirostris, Bestiolina. coreana, B. similis*), Cyclopoid copepods (*Oithona brevicornis* and *Dioithona oculata*) and Harpacticoid copepod (*Euterpina acutifrons*) are being produced in CMFRI. *Apocyclops cmfri* is a promising new species identified from Karwar waters and cultured here.



Temora turbinata



Pseudodiaptomus serricaudatus

Acartia southwelli

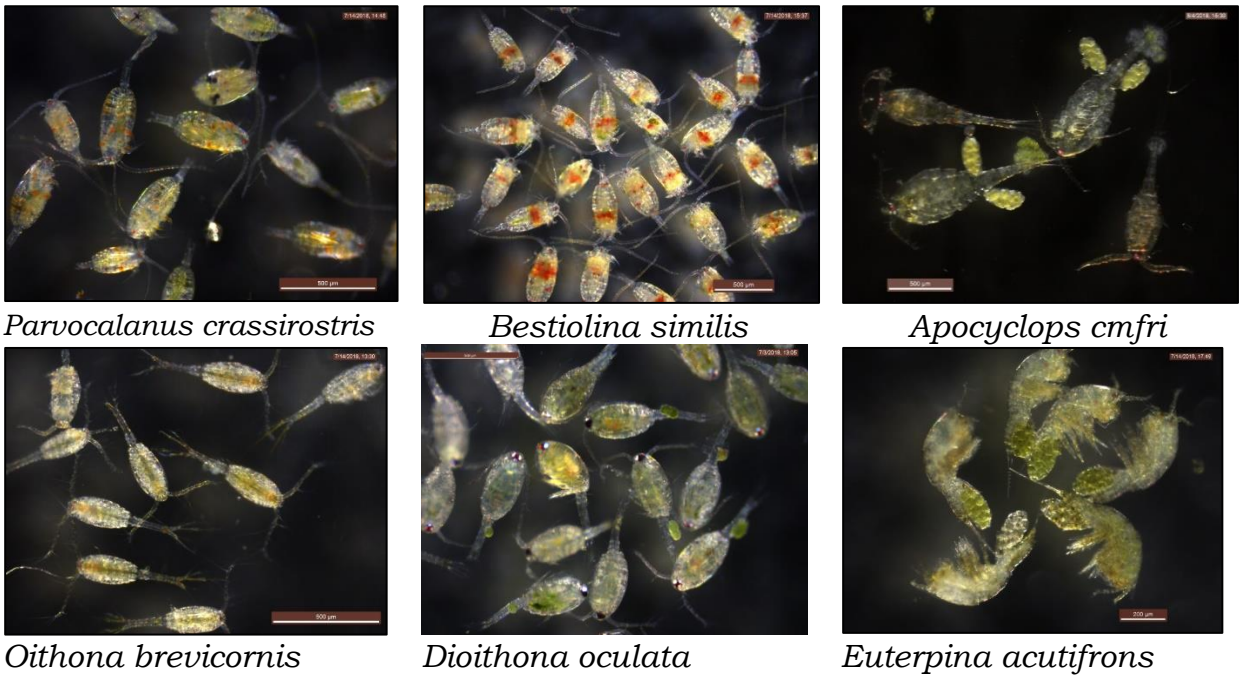


Fig. 1. Copepods developed by CMFRI

Stock culture

The stock culture of microalgae is a prerequisite for copepod culture. Microalgae need to be contamination free. The algal requirement is comparatively less when compared to other live feeds but the purity of the culture is very important. Contaminated algal culture can collapse the stock of copepods. For each species of copepods algae or a combination of algae in the recommended doses are essential.

Stock culture of copepods can be done in tanks of 50-500 litre capacity or even in bigger tanks. Tanks of plastic, HDPE or fibre are ideal. Cement tanks are not ideal. White colour or light colours are desirable because they support visibility. It is easy to assess the population and health of copepods on white background. Cleaning is also easy on white background. In stock culture, the health status of the copepod is more important than its population status.

A few hundred copepods are sufficient to inoculate in stock culture tanks. Tanks attain maximum density within 10-20 days depending on the species cultured. Since production is totally dependent on population, regular harvest is possible even from the stock culture without affecting the total population. Normally the stock culture can continue for 2-3 months with proper maintenance. There are basically no differences in the maintenance of stock and mass culture.

Maintenance of stock culture

On alternate days, the sediments need to be siphoned off from the culture tanks to reduce the ciliate growth and also to maintain good growth of copepods in the culture. The siphoned sediment and water should be kept in 20litre buckets with mild aeration for a few hours for the settling of debris. Live copepods, eggs, and larval forms accumulated in the clear surface of the buckets can be carefully filtered out by passing through a filter of the sieves of the desired mesh size. Copepods collected can be washed and reintroduced in the culture tanks.

The sediment can be diluted again and this process can be repeated several times so that all healthy and live copepods are collected and introduced back into the culture. This process is essential for egg broadcasting species because all the eggs will be settled in the bottom with faecal pellets and wastes. In this way, ciliates and dead organisms can be regularly removed from the tanks. In the case of egg-producing copepods, the filtrate need to be diluted with clear filtered sea water and kept for one or two days with mild aeration. The freshly hatched nauplii can be sieved out regularly using a 30µm sieve and can be introduced back into the culture system. The water level in the culture tank should be brought back to the original level by adding clean, dechlorinated or ozonized and filtered seawater. The tank needs to be washed and reused completely after one month of culture.

Another method of keeping stock culture is without siphoning the bottom for 7 days. Keep on adding feed and maintain water quality parameters without disturbing the culture. After 7 days, remove the bottom debris and transfer the entire culture into another fresh tank. The old tank can be washed, sterilized, and reused.

Mass culture

For the mass culture of 1000-5000 litres, 50-100 litres of inoculum is required. Inoculum needs to be cultured in 1000-litre tanks for inoculating 5 tonne or 10 tonne tanks. Up to 75% of the stock can be used for culture. The inoculum will be ready again within 8-10 days and the tanks will be ready for harvest within 10-25 days period. Thus, a series of tanks starting from 100 litre, 500 litre, 1000litre and 5 tonnes/ 10 tonnes are necessary for establishing a large-scale production system. Care should be taken to increase the volume of water slowly with an increase in population after inoculation, especially in large systems. All tanks should never be filled beyond 75-80% of their capacity. Flat base round drainable tanks are ideal and the water depth less than 1m. Complete indoor tanks also can be used with normal lighting conditions. All tanks should be placed in a bit elevated position so as to assist easy siphoning of bottom samples. Mild aeration is essential in all tanks.

Mass culture can be done as batch culture and continuous culture. For batch culture, the entire tank content can be harvested. For continuous culture, daily harvest is possible from the larger tanks. Most of the species can be cultured in both ways but continuous culture is more successful in egg-broadcasting copepods of the genera *Parvocalanus, Bestiolina, Temora* and *Acartia*.

Maintenance of mass culture

Mass culture tanks of any capacity can be used for large-scale production. Mild aeration needs to be provided accordingly the entire area of the tanks needs to be aerated but there should not be any turbulence or strong flow of water. The inoculum needs to be introduced into 4-5 times higher volume and slowly increase the water level to optimum tank size depopulation of copepods as daily doses. In large volumes of water, unused algae may settle down as debris and can create the development of unwanted organisms. If conditions are favourable within one week, we can reach the maximum capacity of production. In most of the species, we can reach maximum volume within 7-15 days. Depending on the level of sediments, bottom debris needs to be siphoned off from the culture tanks to reduce the ciliate growth and to maintain good growth of copepods in the culture.

The siphoned sediment and water should be kept in buckets with mild aeration for a few hours for the settling of debris. If a large volume of water needs to be filtered, use one or more sieves to collect the sediments. In such cases, an open wide flat tray can be used to reduce the pressure of outflowing water. Live copepods, eggs and larval forms accumulated in the clear surface of the buckets can be carefully filtered out by passing through a filter of the desired mesh size. Copepods collected can be washed and reintroduced in the culture tanks. The sediment can be diluted again and this process can be repeated several times so that all healthy and live copepods are collected and introduced back into the culture. This process is essential for egg broadcasting species because all the eggs will be settled in the bottom with fecal pellets and wastes. In this way, ciliates and dead organisms can be regularly removed from the tanks. In the case of egg-producing copepods, the filtrate should be diluted with clear filtered sea water and kept for one or two days with mild aeration. The freshly hatched nauplii can be sieved out regularly using 30µm sieve and can be introduced back into the culture. The water level in the culture tank should be brought back to the original level by adding clean, dechlorinated or ozonized and filtered seawater.

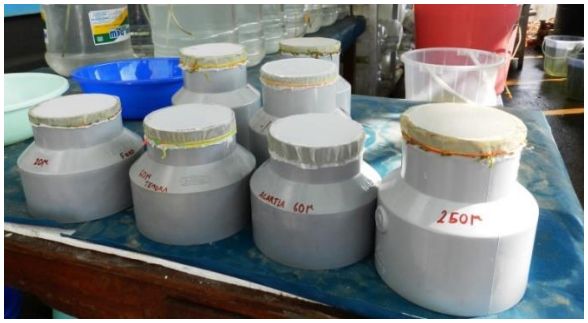


Fig. 2 &3. Sieves of different mesh sizes made from plastic pipe connectors and bolting silk

Fig. 4. Siphoned sediments from the culture tanks kept for isolation of naupliar stages

Fig. 5. Stock culture of microalgae

Problems in the culture

The main problem in culture is ciliate infections in tanks. Overfeeding, fecal contamination, and accumulated debris results in the emergence of ciliates in culture. Ciliates growth can be assessed by the cloudy nature at the bottom of the resident tanks. If care is not given, it will result in the total decline of the population. Prevalence and mean intensity of ciliates in the culture tanks and epibionts on the copepods should be evaluated at regular intervals. *Euplotes* sp. is the most common ciliate in the culture system and *Voticella* sp. is the most common epibiont on the copepods.

The culture can withstand the ciliates up to a certain extent. If ciliates exceed, the culture can be siphoned out and washed through dechlorinated filter water using sieves of 70-8µ and fresh culture should be initiated. The threshold level of ciliates in the bottom water sample of culture tanks is estimated as 7-8nos/ml and if the level exceeds more than 10nos/ml, there can be a sharp decline in the population of copepods in the culture tanks.

The deficiency of feed is another reason for the decline of the culture population. The feed provided should be proportional to the biomass present. If sufficient feed is not provided it will result in adults of some species, especially *Acartia* spp. start feeding on eggs and early larvae which may also lead to the total collapse of the population.

The feed provided should be contamination-free, especially of ciliates. If a mesh of 20 µ is used for filtering the feed, it will help in preventing ciliates to some extent. Pure and mature algae need to be fed for maintaining long-term culture. Immature or collapsed algal feeds shall lead to a decline in population. The settled debris and accumulated wastes in resident sea water is also a substrate for the

development of ciliates and other dangerous organisms. So, the regular renewal of seawater in resident tanks is essential to create a healthy environment for the culture.

Cleaning

A major threat to the copepod population is ciliate infection. The total removal of ciliates is an impossible task. So, by means of proper cleaning, ciliates can be reduced to a large extent. The daily removal of accumulated fecal debris and waste food materials can be done using separate siphoning tubes. In mass culture tanks siphoning can be done on alternate days as described in earlier. The siphoned water has to be collected in separate buckets. The buckets should be contamination free. Later the supernatant portion of the filtrate should be filtered through a 100-micron filter to recover adults if any as mentioned in the earlier section. The very young nauplii collected through a 20µ filter can be washed thoroughly with dechlorinated sea water and can be used for feeding the larvae. Every week, the sides of the culture tanks and bottom should be slowly and carefully wiped using the appropriate brush without disturbing the water. Aeration should be stopped for at least one hour and all the sediments should be allowed to settle down at the bottom. The sediments can be carefully siphoned off and treated in a manner similar to that discussed earlier. As far as possible use all items like sieves, pipes, buckets etc separately for each tank. Enough care should be taken to avoid cross-contamination. Care should be taken for siphoning out the sediment or eggs or copepods. The filter should be placed in a trough and allow the water to overflow through the trough in such a way that the disturbance of the water inside the filter should be minimum.

Hatchery management and protocols for breeding of marine fishes

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INTRODUCTION

Mariculture is the fastest growing sub-sector of aquaculture in the world. In contrast to the global scenario, where mariculture of finfishes is a well-developed industry, in India, it is gradually emerging out from its infancy. Cage as well as pond farming technology is widely recognized as the most important technology in mariculture for increasing fish production to meet the food fish demand. One important aspect hindering the rapid progress of mariculture in the country is the availability of quality seeds of high value fin fishes. However, in recent years, with success in breeding and seed production technology of several high value commercially important finfishes, due to the consistent efforts of ICAR-Central Marine Fisheries Research Institute (CMFRI), the mariculture sector of the country is poised to make a serious contribution to the fish basket of the country. Presently, quality seeds are available for cobia, Indian and silver pompano, grouper, snapper and sea-bream round the year in various hatcheries of CMFRI at multiple locations.

Marine finfish seeds are generally produced in the controlled conditions with various indoor facilities commonly known as hatchery. Marine finfish seed production required various steps, which are interlinked and interdependent to produce quality seeds for the culture activities. Hatchery management is nothing but to interconnect these complex and wide-ranging set of tasks collectively to achieve sustainable production of quality seeds. The present chapter aims to provide a general view of the hatchery management and breeding protocols of marine finfish with special reference to orange spotted grouper (*Epinephelus coioides*) and Indian pompano (*Trachinotus mookalee*). The quality of seed stock depends on a number of factors. These include physical status of the broodstock such as size, age, level of maturity, number of spawns per year, appearance and health condition; and these are also largely dependent on broodstock conditioning practices. In addition, genetic quality of the broodstock also impacts significantly on the quality of the seed-stock. From an aquaculture perspective, hatcheries in the long-term should aim at consistently providing good quality seed to the nursery or grow out sectors through proper husbandry practices; and ensuring long-term genetic quality of the broodstock with a sound genetic management plan.

BROODSTOCK DEVELOPMENT

Acquisition

Initially, broodstock can be acquired through collecting or purchasing wild fish. Record maintenance of collected adult fish being brought into the hatchery for

broodstock development programme is important and need to be maintained for future use.

Orange spotted grouper are diandric protogynous species where males are either derived from a juvenile phase or the transition of post; spawning females. However collecting males from wild is very difficult. While collecting adult orange spotted grouper at Visakhapatnam RC of CMFRI, Visakhapatnam, the smallest recorded size of mature male of orange spotted grouper from wild was 18 kg. Handling of such bigger sized male is problematic; hence collecting smaller fishes of around 2.0 kg is good for developing male broodstock by manipulating sex using different hormones and enzymes.

Fig. Orange spotted grouper

Fig. Indian pompano

Adult Indian pompano of more than 3 kg can be collected from the wild by hook and line and transported to the hatchery. The sexes are separate and the maturation size is around 3.0 kg, thus the same size fish needs to be collected in more number for developing broodstock.

Pond or cage cultured fish is another source of broodstock. Cage, pond or tank-reared fish are already accustomed to culture conditions and consequently easier to develop into suitable broodstock. However, it can take 2 years to grow juvenile fish up to broodstock size, and more over inbreeding might happen when collecting fishes from culture system for developing broodstock. While selecting a fish for developing broodstock, few characteristics needs to be kept in mind

- Normal body shape and colour
- Absence of skeletal deformities
- Overall health status, i.e. devoid of large wounds, haemorrhages, infections and parasites
- Normal behaviour, such as a good response to feed, controlled buoyancy to maintain position in the water column
- Best growth and feed conversion rate within its age group when we are selecting from cultured one.

Transport

Generally adult fish should be collected from nearby area as far as possible. The collected fish should be transported in covered tanks containing aerated or oxygenated water to reduce stress. Mild sedation, using approved sedatives for fish, such as 2 phenoxyethanol @ 50 ppm can be used to reduce stress and make handling the fish easier and safer. If the fish is collected from the culture system, they should not be fed for the previous 24 h to avoid deterioration of water quality during transportation. Generally, fish regurgitate the feed if they are handled after feeding, thus it will spoil the water quality as well as induce stress to the collected fish.

Treatment after collection

Once the fish are transported to the hatchery, the fish need to be shifted in quarantine area. It is advisable to quarantine them to reduce the parasitic or bacterial infection. Generally the quarantine period varies from 3 to 4 weeks and can be carried out in small tanks of 1 m3 to facilitate easy handling. Groupers are demersal fish and hence after catching, the fish from wild shows barotrauma. Barotrauma occurs due to the differences in the pressure from where they are caught and the sea surface. Due to differences in pressure, air bladder fills with air and bulges and gives stress to the internal organs. This needs to be relieved by inserting a needle in gas bladder through anus. Once the fish are swimming normally with controlled buoyancy to maintain position in the water column, they need to be shifted in appropriate tank. The wild collected orange spotted grouper should be given bath treatment with formalin at the rate of 200 mg L-1 for 30 min followed by 5 min, dip in freshwater. Fish should be shifted to another tank after the treatment with fresh seawater. This treatment should be repeated every 4 to 5 days for at least 6 times. In case of Indian pompano, the fish should be treated by giving bath treatment of formalin in freshwater at the rate of 30 mg L-1 for 15 min. Fish should be shifted to another tank after the treatment with fresh seawater. This treatment need to be repeated every 4 to 5 days for at least 8 times consequently. Once the fishes are free from the parasite, the fish can be shifted from quarantine area to broodstock tank.

Broodstock tanks

Generally broodstock tanks are used for culture and maturation of broodstock as well as for spawning. Due to the size of the broodstock and the natural spawning behavior of the fish, larger tank of 50 -100 m3 are preferred for broodstock tanks. At Visakhapatnam Regional Centre of ICAR-CMFRI, 125 m3 concrete reinforced tanks with water volume of 100 m3 are used for broodstock development cum spawning. Generally tanks should be round, or square or rectangular with rounded corners, however round tank is preferable. Medium-range blue, green or grey colour is preferable for the broodstock tank. Tanks should be at least 2.0 m deep and preferably 2.5 m to allow sufficient room for spawning behavior of orange spotted grouper, which involves pairs or group of fish swimming upward from the tank bottom while releasing ova and sperm. However, in case of Indian pompano, tank of smaller size of 20-30 m3 can also be used for broodstock development. Generally the broodstock tanks are used as flow-through system, however re-circulating aquaculture system are better for broodstock development and spawning. An overflow pipe is fixed 15 cm down from the surface of the broodstock tank to the egg collection tank. The egg collection chamber is installed with 500 µm mesh for sieving the eggs. The egg collecting chambers are connected with the re-circulating loop so that the water is pumped back to the broodstock tank. It is advisable that broodstock area should be roofed in order to reduce the growth of algae on the tank wall and bottom, which makes egg collection difficult and increases the risk of failure of re-circulating system. Moreover, dirty tanks need to be cleaned frequently which may stress the broodstock and cause spawning failure or lower the quality of eggs. Broodstock tanks should have re-circulating facilities with 300% water re-circulation. Sea water used for broodstock development should be filtered and clear with stable salinity of 30-35 ppt and water temperature of 27-32^{o}C.

Broodstock management

Gender identification

The male and female of orange spotted grouper and Indian pompano cannot be identified morphologically. The sex of individual fish can be confirmed only by physical examination i.e live ovarian biopsy (LOB). Fishes need to be anaesthetized by using 2 phenoxy ethanol at the rate of 200 ppm for 2-3 minutes. The anaesthetized fish is then cannulated using the fish cannula or baby feeding tube CH 6 having an inner diameter of 1 mm and an outer diameter of 2 mm. The cannula is inserted into the urinogenital orifice of males and the oviduct of females. Fish to be cannulated are anaesthetised and a wet cloth or towel is placed over the eyes to assist in calming the fish.

The cannula is guided into the fish for a distance of 6–7 cm and suction is applied to the other end of the cannula as it is withdrawn. After withdrawal, the sample within the cannula is expelled onto a microscope slide for immediate examination or into a vial containing 1% neutral buffered formalin for later measurement of egg diameter.

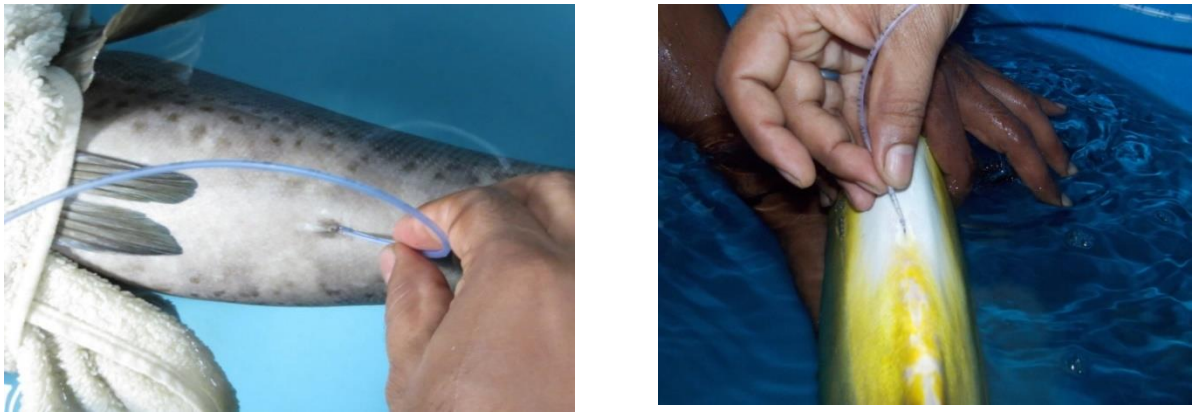

Fig. Cannulation of orange spotted grouper and Indian pompano

Generally, females of orange spotted grouper and Indian pompano in spawning condition will have oocytes in range of 400–500 and 500-600 µm diameter respectively. To confirm matured male, the abdomen of an anaesthetised fish is gently massaged in a head-to-tail direction. A sexually ripe male spawner will extrude copious milt from its urinogenital pore. If no milt is expressed, the fish is immature, a male not in spawning condition. Once the sexes of the fishes are confirmed, they are tagged with passive integrated transponder (PIT) and the tag number need to be maintained for future use.

Sex-reversal

As noted earlier, orange spotted grouper are diandric protogynous, and getting male from wild is very difficult; thus a hatchery manager needs to manipulate the sex by using hormones and enzyme. The fifty percent of the stocked and acclimatized fishes should be implanted with pellet containing 17 α methyl testosterone and letrazole at the rate of 5 and 0.2 mg kg-1 body weight respectively (Ranjan *et al., 2015).* The pellet can be prepared using gum acacia, cholesterol and 17 α methyl testosterone in the ratio of 1:2:1. These chemicals need to be weighted accordingly, and mixed with few drops of water, which then form dough and is

prepared in required size as per the implanter. This prepared pellet needs to be implanted on dorsal side of the brooders below to the dorsal fin in musculature. Before implantation, fish should be anaesthetized to avoid handling stress. This hormonal dose will convert the female to male within 2 months.

Feeding

At Visakhapatnam RC of CMFRI, broodstocks are fed to satiation at least once daily in the morning with fresh or frozen squid for orange spotted grouper and squid along with clam meat for Indian pompano. Various vitamins namely, vitamin A (25,000 IU, USV limited, Nani Daman, India), vitamin B-complex (Pfizer, India), vitamin C (500 mg; Abbott Healthcare Pvt. Ltd., Thane, Maharashtra, India), vitamin E (400 mg) (Merck, Goa, India) and vitamin–mineral mix (Agrimin Forte, Virbac Animal Health India Pvt. Ltd., Mumbai, Maharashtra, India) are supplemented twice a week along with the feed to avoid any possible nutritional deficiencies. These vitamin premix tablet are inserted inside the squid and fed one by one till the satiation of the fish.

Tank cleaning

If the broodstock tank is connected with re-circulating system, faeces and other waste material such as dead eggs and feed remains will not accumulate in the tank. The broodstock tank doesn't require any cleaning; however it is advisable to clean the tank at least once in a year by removing all fish from broodstock tank.

Spawning/ Induced spawning

Orange spotted grouper broodstock are allowed to spawn naturally in the tanks. If the tank is stocked with matured female (2.0 kg) and hormone pellet implanted male, spawning will start from 3rd month onwards. Spawning generally occurs at dusk (3 pm – 6 pm) for ten to fifteen times in each month. At Visakhapatnam RC of CMFRI, grouper broodstock spawn throughout the year. During the spawning period, orange spotted grouper may spawn between 0.8 and 6.0 million eggs each time. The re-circulating system needs to be stopped by 15.00 h during winter and 17.00 h in summer to avoid washing out of eggs. If the spawning happened then, the re-circulatory system should be in OFF till the next morning when the eggs are being collected. Otherwise, if the spawning did not happen by 18.00 h, the re-circulating system should be ON.

In the case of Indian pompano, natural spawning is rare and in most situations, needs to be stimulated with the use of hCG or LhRH hormones for the spawning. Once the female ova size exceeds 500 µm, and the males are oozing, then the both sexes needs to be injected hCG at the rate of 350 IU/kg body weight, or LhRH at the rate of 100 and 50 µg/kg body weight of female and male respectively. The fishes for cannulation should be anesthetized to avoid stress during the cannulation and injection. As far as possible, avoid the checking of male before inducing for spawning. The fishes are then shifted back to the same tank for spawning. The fishes respond after 36 h of injection at a temperature range of 28-32 oC. The re-circulating system needs to be stopped after 35 h of induction to avoid washing out of eggs. Generally, the Indian pompano will respond in night between 23.00 to 24.00 h

Egg-handling

Collection

The fertilized eggs of orange spotted grouper and Indian pompano are floating in nature at a salinity of more than 30 ppt. This floating nature of eggs is helpful in collection by overflowing the egg to the collecting chamber. The fertilized eggs of orange spotted grouper and Indian pompano are non-adhesive and pelagic, and range from 0.8 to 0.9 mm and 0.9 to 1.0 mm in diameter respectively. A 500 µm mesh size bag is tied in egg collecting chamber, which is connected to broodstock tank via a 3 inch PVC pipe. The water level in the broodstock tanks should be raised and the re-circulating system should be started in the morning at 6.00 h by switching on the water flow from the egg collecting chamber to the re-circulating system. The eggs collected in egg collecting chamber are scooped with the help of 500 µm mesh size sieve and transported to the larval rearing section of hatchery. The eggs are sensitive to handling stress during the early embryonic developmental stages and should only be collected after the embryo has developed optic vesicles, i.e. eyed stage. Handling or iodine treatment of eggs before this stage will lead to increased mortality and higher incidence of failure of hatching.

Disinfection

The collected eggs should be disinfected with 20 ppm active iodine for 10 minutes with strong aeration. The treated eggs are then washed with deionized sea water and stocked in glass aquarium with water salinity of 30-32 ppt. As noted earlier, fertilized eggs will float on the water surface whereas unfertilized or dead eggs will settle in the bottom, , which needs to be removed by siphoning the bottom of the aquarium. Only floating eggs on surface are used for larval rearing because these are more likely to be of good quality than sinking eggs, which are unfertilized or dead or inferior of quality.

Egg quality

Egg quality in marine finfish is generally evaluated using both qualitative and quantitative methods.

Qualitative evaluation of the eggs

Fertilized eggs are examined under a microscope (4x or 10x) for qualitative evaluation of embryos. The good fertilized eggs will have following characters:

- Eggs should be regular in shape
- During the early stages of embryonic development, the individual cells should be regular in size
- Eggs and embryos should be transparent, with no dark areas
- Chorions (eggs hells) should be free of any parasites or fouling organisms.

In case of orange spotted grouper, if there is only a small proportion of eggs with irregular shape, dark or with aberrant embryonic development, then the eggs can be used in the hatchery. However, if the proportion of eggs exhibiting abnormal characteristics (>10%) is high, the entire batch should be discarded, as there is very less chance of larval survival during the larval rearing period. The eggs should be discarded when they have parasites or fouling organisms attached to them for the probability of transferring pathogens to the hatchery.

Quantitative evaluation of eggs

Fertilization and hatching rates are also used as indicators of egg quality. These rates for groupers are higher than 50%, and preferably more than 80%. Grouper larvae from batches of eggs with low fertilization and hatching rates (<30%) are regarded to be of poor quality. Poor quality larvae exhibit low survival with a high incidence of deformities and other health problems and are usually discarded.

Fertilization rate

Estimation of fertilization rate is undertaken several hours after fertilization has taken place, but well before hatching, as because embryonic development makes it easier to discriminate the fertilized eggs from the unfertilized eggs. For estimating fertilization rates, at least 10 egg samples are taken to have an accurate estimate. Before taking the sample, the eggs should be mixed properly so that the fertilized and unfertilized eggs have proper representation.

The number of fertilized eggs and unfertilized eggs present in the samples are counted. Total number of eggs present in the sample is calculated by adding the numbers of fertilized eggs and unfertilized eggs of the sample. The numbers of fertilized eggs are divided by total numbers of eggs and multiplied by 100 to obtain fertilization rate (%). The fertilization rates of orange spotted grouper and Indian pompano is generally more than the 80% and 70% respectively at Visakhapatnam RC of CMFRI.

Hatching rate

The stocked fertilized eggs usually hatch out 18-20 hrs after fertilization at a water temperature range of 28-30oC. Estimation of the hatching rate is undertaken when hatching is completed. The hatched out larvae drift on the water surface. The estimation of hatching rate is performed from random vertical water samples of hatching tank using PVC pipe from 10 different places. The volume of sampled water and number of larvae in it is estimated. The total number of larvae present in the hatching tank is calculated by extrapolating the sampled estimate for the whole tank. The total number of hatched out larvae are divided by the total number of fertilized eggs stocked and multiplied by 100 for calculating hatching rate (%). The hatching rate of orange spotted grouper and Indian pompano at Visakhapatnam RC of CMFRI is more than 85% and 80% respectively.

Stocking of larval rearing tanks

Generally, fertilized eggs are stocked at the later of eyed stage because they are more robust than the newly hatched larvae. Newly hatched larvae are very sensitive to physical shock or changes in water quality, and moving them to the larval-rearing tanks may result in high levels of mortality. Because the hatching rate is not known before the eggs are stocked in the larval-rearing tanks, the number of eggs to be stocked needs to be estimated using historical hatching rates for that hatchery. Accurate estimates of the number on larvae stocked can be back-calculated using data from the actual batch stocked, as described above. If hatching rates are low, the larvae in the larval-rearing tanks should be discarded, and the tanks cleaned and disinfected.

Broodstock development, breeding and seed production of Seabreams and Rabbit fish

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Introduction

Nearly half of the fish consumed worldwide comes from aquaculture. Of the overall fish production in the world a total of 156 million tonnes were used for human consumption, which equates to an estimated annual supply of 20.5 kg per capita and around 17% of global animal protein consumption and 7% of total protein consumption (FAO 2020). Due to rising population and food consumption as well as ongoing global degradation of the environment (land, water, and the climate) food security is crucial. Aquaculture production need to be enhanced further in order to meet the demands of the growing population which will lead to improve nutrition and food security for all communities.

The essential amino acids, vital fats including omega-3 fatty acids, vitamins and minerals are present in ample quantity in fish, which is a good source of protein. Consumption of fish provides many health benefits. Health experts also suggest that fish in the diet are beneficial to pregnant or nursing women and children. Fish consumption is steadily increasing, feeding billions of people and ensuring diets that are nutrient-dense is a greater challenge for aquaculture. Availability of high-value fish to local/ domestic markets can be assured through aquaculture. Fishes are available in large variety compare to other food systems. Aquaculture can provide economical and nutritious food for the world's poorest people, whose diets lack most of the essential nutrients. Fish is currently the primary protein source for millions of people, especially in developing countries. Aquaculture will be the major fish producing sector in future, which ultimately ensures good health and wellbeing of human beings.

Aquaculture production can be enhanced only through adopting innovative technologies and by introducing new candidates for farming. ICAR-CMFRI has successfully standardised the farming of marine fishes such as Cobia (*Rachycentron canadum*), Silver pompano (*Trachinotus blochii*), Indian Pompano (*T. mookali*) and Orange Spotted Grouper (Epinephelus coioides) by introducing the standard hatchery technologies for these commercial fishes. Adding to this table, recently Karwar Regional Station of ICAR-CMFRI has contributed to the diversification of farmed species by standardizing the seed production of few locally available commercially important seabreams and rabbit fish.

The maze rabbit fish/ vermiculated spine foot, *Siganus vermiculatus* is one among the largest economically important fish suitable for farming in in cages, ponds and tanks. These herbivores fishes can substantially reduce the cost of production in commercial farming due to it's low protein requirement and it fetches an average market price of Rs. 250-300/ kg in domestic market. Moreover they are more compatible for polyculture since they can effectively control bio-fouling in cages.

The station has also developed captive breeding of picnic seabream (*Acanthopagrus berda*). It is the first of its kind achievement in the country. Also known as black seabream and goldsilk seabream, the fish is high in demand having high recreational value, excellent meat quality and high economic value. The fish is an excellent species for mariculture owing to its faster growth rate, strong resistance to diseases and ability to cope up with wide variations in environmental parameters such as salinity and temperature.

Karwar Regional Station has succeeded in the induced spawning of the newly described sparid fish, ***Sparidentex jamalensis*** Amir, Siddiqui & Masroor, 2014 which has been reported first time from Pakistan waters. The species has been recently identified and reported from Calicut, also. The availability of this commercially important sparid species at Karwar also was confirmed last year, employing conventional and molecular taxonomy by ICAR-CMFRI.

Seed production of seabream

Seabreams are prominent group of farmed fishes globally, that can be reared for its recreational value, excellent meat quality, market acceptance, high economic value. But majority of them exhibits varying degrees of hermaphroditism which makes their breeding in confinement more complicated due to asynchronized maturation of different sexes. Picnic seabream or black seabream, *Acanthopagrus berda* is an important fish in fisheries and aquaculture because of its high recreational value, excellent meat quality, high economic value, ability to tolerate wide variations in environmental parameters such as salinity, temperature, strong resistance to diseases and faster growth rate. Therefore, *A. berda* in tropical Indian waters has the potential to attract commercial interests in the near future. Black seabream is carnivorous in nature and is a Protandrous hermaphrodite. It is a schooling species feeding mainly on echinoderms, worms, crustaceans, molluscs and small fishes. The fishes of genus *Acanthopagrus* have good consumer acceptability due to their excellent meat quality. Approximate price for the fish in local market in India is Rs.470/ kg. (US $ 7/Kg).

Fig. 1. *Sparidentex jamalensis* adult *and Acanthopagrus berda* adult

Broodstock development of seabream

Live fishes collected as commercial catch from Kali estuary, Karnataka, India using cast nets were transported to the marine cage farm of Karwar Regional Station of ICAR-Central Marine Fisheries Research Institute, Karwar, Karnataka. The fishes were quarantined and disinfected with $KMnO_4$ solution dip (50 ppm for 2 min) before stocking in the marine cage farm of the station. The fish collection consist of a mixture of *A. berda* and *S. jamalensis.* The fishes collected for broodstock development were maintained in a 6 m diameter Galvanised Iron (GI)

circular cage at the marine cage farm. The fishes were reared providing low value fish as feed @ 5 % of body weight. Vitamin (E and C) mix (1 g per kg wet feed) and cod liver oil (10 ml/ kg wet feed) were provided along with the feed twice in a week. The daily ration was divided and given twice daily (10 am and 5 pm). Periodic net exchange was done for the proper maintenance of the cage. Water quality parameters were recorded using a multipara meter water quality analyser. One month prior to the breeding trials the fishes were fed with maturation feed such as raw squid and oyster meat at 3 % of body weight.

Fig. 2 Broodstock development of seabreams in marine cages.

Selection of brooders for induced breeding of seabream

Observations on the maturity of the fishes was done once in a month for both males and females. For males the presence of oozing milt on applying pressure on the belly was checked. For females ovarian biopsy was carried out using a catheter with a diameter of 1.6 mm to 2 mm (Feeding tube HC 6 or 7, India) depending on the size of the fish. The biopsy samples can be analysed using a stereozoom microscope (Onix Vision, India) with digital imaging facility.

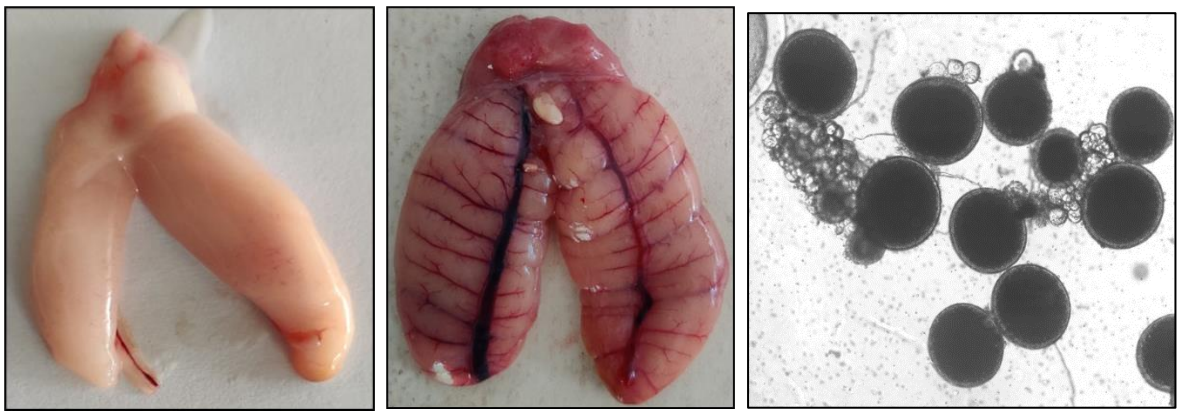

Fig. 3. Mature testis and ovary of *A. berda* and ovarian biopsy of matured oocytes

Induced breeding of *A. berda*

Standardisation of breeding and seed production of this species has been achieved for the first time in India at the Karwar during February 2021, employing induced breeding techniques using Salmon GnRH –Analogue hormone (OVAFISH, Bhoomi Aqua International, India) as the inducing agent. The hormone was admistered in single dose for both male and female at 0.5 ml / kg fish or in multiple doses. Fishes spawned after 36 hours of inducement and the pelagic eggs hatched after 22 to 24 hours at a temperature of 28 to 30 ^{0}C. Fecundity was 0.25 million

per female (450 g) and 86% of the eggs hatched after 24 hrs. Larval rearing was carried out in 100 litre tanks with various live feed organisms such as Copepods, Rotifers and Artemia; stocking 100 eggs per litre. Metamorphosis of the larvae initiated in 24th day post hatch (DPH) with 9 % survival in a tank. This is the first report of the breeding of a seabream from Indian waters.

Table 1. Feeding chart for larval development of *A. berda*

Larval stage (*dph)	Micro algae (3 x 10^5 cells per ml)	Copepod nauplii (20 to 50 µm) (3 nos / ml)	Rotifer (50 to 200 µm) (5 nos / ml)	Artemia (10 nos / ml)	Micro-capsulated feed I (0.5 g/ tank/ day)	Micrc capsı feed g/ tar
0						
1						
2						
3						
4						
8						
8 -10						
10 -15						
15-20						
20 -25						
25 -30						
30 -35						
35 -40						
40 -45						

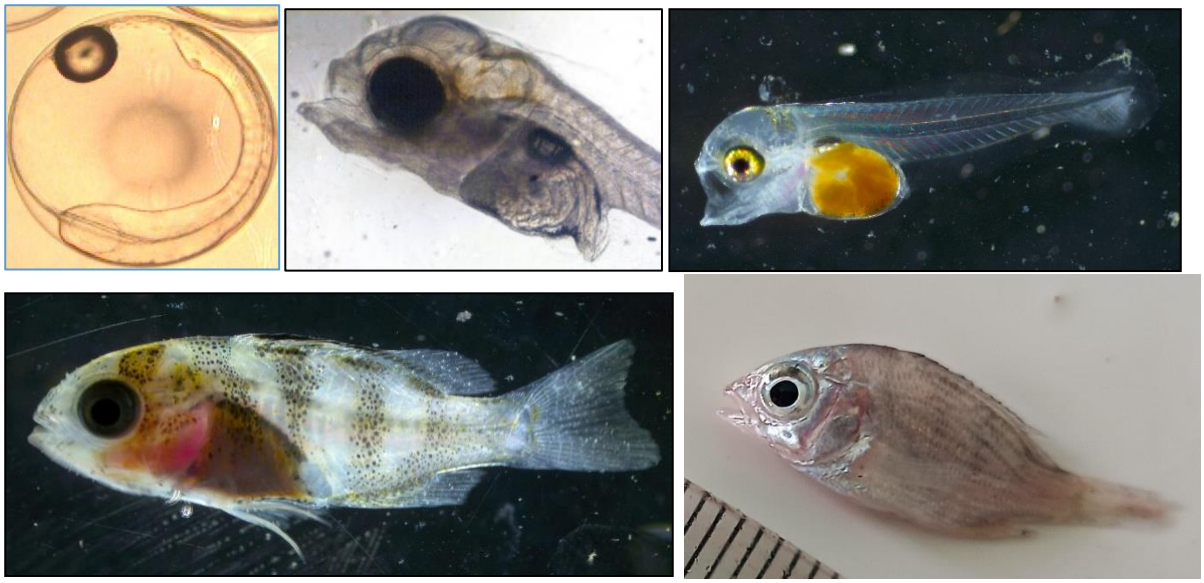

Fig. 4. Various developmental stages of A. berda larvae

Breeding of *S. jamalensis*

Periodic microscopic observation of ovarian biopsy indicated progressive gonadal development in the broodstock. The broodstock developed for this

protandrous hermaphrodite consisted of females having maturing oocytes and males with oozing milt during the breeding season. Hormonal inducement was achieved for final maturation and spawning of the female using cholesterol based LHRH analogue pellets (50 µg / kg fish) and the males were not induced. Pelagic fertilised eggs with a single oil globule were obtained with an absolute fecundity of 0.42 ± 0.06 million / kg fish and a fertilisation rate of 82 ± 2.4 %. Eggs hatched after 18 to 20 hr with 80 ± 4.6 % hatching rate at 28 ^{0}C. The newly hatched planktonic larvae measured 1.8 ± 0.12 mm in total length. Mouth opening of the larvae was observed on 2nd day post hatch (dph). Resorption of the yolk sac was observed on 3rd dph.

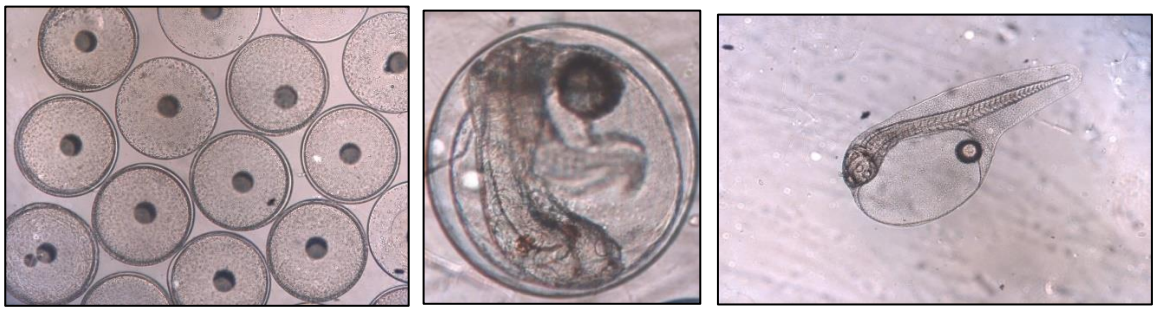

Fig. 5. Fertilised egg, embryo and larvae of *S. jamalensis*

Breeding of Rabbit fish

Siganids are widely distributed in Indo-West Pacific region and Mediterranean region. They occupy low on food web and can be grown on a low protein diet. In captivity, they show omnivorous feeding behavior and feed on a variety of food items. They can be raised on artificial feed and hence amenable for culture. *Siganus vermiculatus* is one among the siganids which can reach above 2 Kg weight. They are fast grower compared to other siganid species and are tolerant to wide ranges of salinity, temperature, and pH. Their tender and delicious meat, local preference, growth rate, tolerance to environmental factors and low protein feed requirements makes them an ideal candidate for mariculture sector. The fish is reported to follow lunar periodicity. Our study is the first report of induced breeding of *Siganus vermiculatus* during full moon phase of lunar cycle and first report of successful larval rearing to metamorphosis in India.

Fig. 6 Broodstock of *S. vermiculatus* and the broodstock management in mini-RAS

Broodstock development

The wild collected fishes were reared in 6m diameter galvanised iron (GI) cages. They were stocked at 1/m^3 and were fed formulated floating pellets enriched with vitamin E, vitamin C, cod liver oil, soy lecithin @ 2% body weight. Matured female having an average ova diameter of 440 microns along with a male fish with thick oozing milt was selected for breeding. Pilot farming of 500 nos. of *Siganus vermiculatus* was carried out in a mini RAS of 10 ton capacity using pellet feed. Fishes recorded an average growth rate of 0.58 g/day with a survival of 88%.

Induced breeding

Fish were induced to spawn in hatchery by intramuscular injection of human chorionic gonadotropin (HCG) @ 500 IU/day and 200 IU/ day respectively for 2 days at an interval of 24 hrs. Spawning occurred on the next day after the night of full moon within 20-21:30 h of second injection. An overall fecundity of 18480 ± 18307.4 was estimated with 68% - 71% of viable eggs and hatching rate of 73% - 85%. Green water system with microalagae, *Nanochloropsis salina* and *Isochrysis galbana* were employed for egg incubation cum larval rearing tanks. The fertilised eggs (0.57 ± 0.02 mm) hatched out between 24 hrs to 25 hrs at a salinity of 31.55-32.25 ppt and temperature of 29.3-30.8^{o}C. The newly hatched-out larvae measured 1.92 ± 0.08 mm in total length with a transparent yolk sac (0.63 ± 0.04 mm) and an oil globule (0.23 ± 0.02 mm). Mouth opening of 89.03 ± 9.4 µ was observed by 42 hrs post hatch. A feeding regime with copepod nauplii, *Parvocalanus crassirostris* as the first feed was used. Copepodites, enriched rotifers, *Artemia* and artificial pellet feeds were also used during larviculture. Larvae attained metamorphosis during 35-37 DPH and the post larvae with vermiculated pattern measured 2.52-2.84 cm and 0.28-0.30 g. The larval rearing protocol realized a survival of 4.2% - 6.1 %.

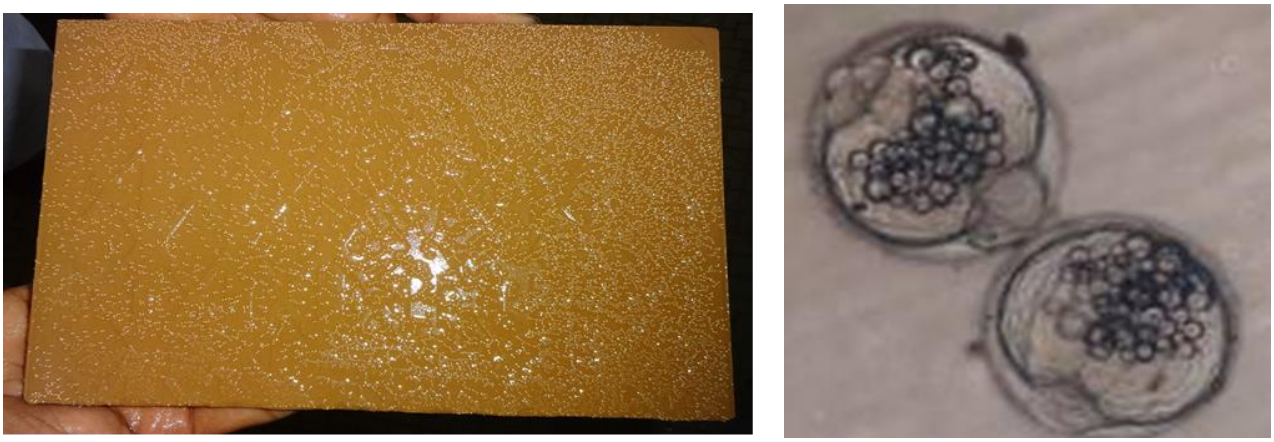

Fig. 7. Fertilised egg of S. vermiculatus attached to substratum and the magnified view of fertilised egg

Days post hatch	Frequency	0	1	2	3	4	5	6	7	8-9	10	11-14	15-17	18-20	21-27	28	29-32	33-37
Microalgae (2-3 x 10^5 cells/ml)	M																	
Parvocalanus crassirostris adults (0.5 no./ml)	M &E																	
P. crassirostris nauplii (1-2 no./ml)	M &E																	
P.crassirostris copepodites (2 no./ml)	M &E																	
S type rotifer (<100µ size, 8-10 no./ml)	M &E																	
S type rotifer (100-200µ size, 10-15 no./ml)	M &E																	
L type rotifer (<150µ size, 5-10 no./ml)	M &E																	
L type rotifer (<150-300µ size, 10-12 no./ml)	M &E																	
Artemia (Umbrella stage, 0.5-1 no./ml)	M &E																	
*Artemia (*enriched, 1-2 no./ml)	M &E																	
Artificial feed (Prince wean, 50-150 µ)	M, A & E																	
Artificial feed (150-550 µ)	M, A & E																	
Bottom Siphoning																		
Water Exchange																		
10%	M &E																	
30%	M &E																	
50%	M &E																	
100%	E																	

Fig 8. Feeding chart for larval development of *S. vermiculatus.*

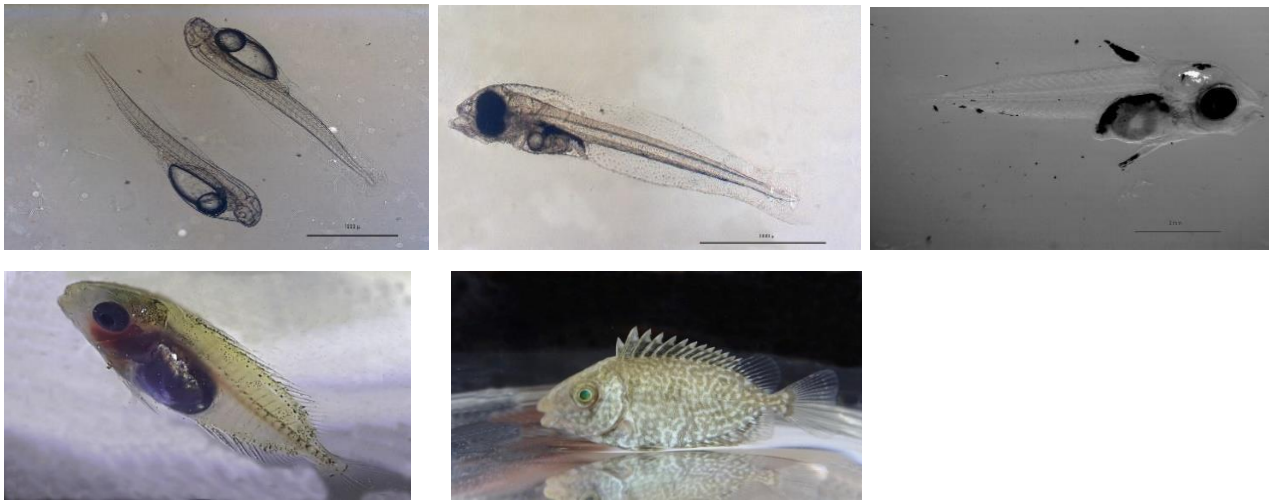

Fig. 9. Various developmental stages of *S. vermiculatus* larvae

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Mariculture Technologies in India

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A globally competitive, technologically appropriate and diverse mariculture sector that meets increasing demand for seafood and products that are affordable and meet high standards for safety, quality, and environmental stewardship, with maximum opportunity for profitability and economic growth is the need for the coming years. Many challenges the mariculture sector has to face are in combating diseases, broodstock improvement and domestication, hatchery production of more species, development of appropriate feeds, supplementation of fishmeal and fish oil, feeding mechanisms, innovative grow-out technologies and water-quality management.

Mariculture involves the cultivation of marine organisms in seawater for food and other products either in the open ocean, an enclosed section of the ocean, or in tanks, ponds or raceways. About 600 aquatic species are cultured all over the world in a variety of farming systems and facilities of varying input intensities and technological sophistication. Mariculture activities other than for human consumption include live bait farming for fishing, live ornamental animal and plant species and ornamental products (pearls and shells), fishes cultured as feed for certain carnivorous farmed species, culture of live feed organisms such as plankton, Artemia and marine worms for use as feed in hatcheries and grow-out systems, aquaculture hatchery and nursery outputs for on-growing in captivity or stocking to the wild and capture based aquaculture. Asia accounted for 89% of world aquaculture production by volume in 2010, up from 87.7% in 2000. Mariculture represents an opportunity to provide a sustainable supplement to the marine capture fishery.

Mariculture has a relatively long history, while, the modern intensive mariculture is only 35 years old, producing a steadily increasing proportion of the world's seafood during this period. Aquaculture production currently makes up almost half of the marine capture fisheries. Moreover, aquaculture production has more than doubled over the last fifteen years and this trend is continuing whilst traditional fishing production is declining as a result of overexploitation. But aquaculture, both in inland waters and marine and coastal areas, has problems, including habitat degradation, disruption of trophic systems, depletion of natural seed-stock, transmission of diseases, and reduction of genetic variability. To solve these problems it is needed to diversify aquaculture and improve its sustainability. In particular, we need to better understand possible interactions between mariculture and natural environments to minimize the potential for habitat degradation, introduction of invasive alien species, etc.

Sea cage farming

The cage culture which initiated in Norway during 70s got developed into a high tech industry in many countries all over the world for high value fishes. The major advantage in countries where cage culture has been commercialized is that they have large, calm and protected bays to accommodate the cages safely against any unfavourable weather conditions. Recent technological advance is in cage design and with new species. In the past, the industry had typically used steel-framed rectangular support structures for the net cages, with walkways around them as work platforms. After that HDPE cages came into existence. Of recent submersible cages which can overcome rough oceanic conditions are being designed and used. Farming was mostly in the inshore waters and since the commercial aquaculture of marine finfish will continue to expand in future, it will take place more in offshore locations than have traditionally been used. By integrating the cage culture system into the marine aquatic ecosystem, the carrying capacity per unit area is optimized because the free flow of current brings in instantaneous exchange of water and removes metabolic waste and excess feed. Thus economically speaking, cage culture is a low impact farming practice with high economic returns and with least carbon emission activity. In view of the high production attainable in cage culture system and the presence of large sheltered coastal waters in many countries, marine cage farming can play a significant role in increasing fish production.

Recirculating Aquaculture Systems (RAS)

A notable technology-based development in the farming sector has been a significant expansion in fish production using closed-recirculation systems. Recirculation aquaculture systems (RAS) are systems in which water is (partially) reused after undergoing treatment. Each treatment step reduces the system water exchange to the needs of the next limiting waste component.RAS have been developed to respond to the increasing environmental restrictions in countries with limited access to land and water.RAS offer advantages in terms of reduced water consumption, improved opportunities for waste management and nutrient recycling and for a better hygiene and disease management, biological pollution control (no escapees), and reduction of visual impact of the farm. These systems are sometimes referred to as 'indoor'or 'urban' aquaculture reflecting its independency of surface water to produce aquatic organisms. In addition, the application of RAS technology enables the production of a diverse range of (also exotic) seafood products in close proximity to markets, thereby reducing carbon dioxide (CO_2) emissions associated with food transport.

Integrated Multi-Trophic Aquaculture (IMTA)

Integrated multi-trophic aquaculture (IMTA) refers to the explicit incorporation of species from different trophic positions or nutritional levels in the same system. IMTA has been defined based on studies in marine habitats involving joint aquaculture of fed species, usually fish, together with extractive species such as bivalves and/or macroalgae. IMTA can also allow an increase in production

capacity for harvesting of a particular site when regular options have established limitations.

Ecosystem approach to aquaculture (EAA)

In recent years, FAO has been working on the implementation of the ecosystem approach to aquaculture (EAA) as a way to improve the governance of the sector; an Integrated mariculture – A global review ecosystem approach to aquaculture is a strategy for the integration of the activity within the wider ecosystem in such a way that it promotes sustainable development, equity and resilience of interlinked social and ecological systems. The EAA promotes the efficient use of nutrient resources as well as the opportunity of diverse products and benefits (and beneficiaries) while reducing impacts, and therefore integrated aquaculture becomes a very important practical way to implement such an approach.

The future expected increases in energy prices, costs for aquafeeds and the strengthening of environmental regulations should facilitate the implementation of integrated systems. However, if integration of e.g. fed species with extractive species (e.g. filter feeders, seaweeds) results in beneficial environmental effects – either locally through waste remediation or at a larger scale with respect to efficiency in resource utilization – such services should be internalized in order to benefit society as a whole (e.g. such as waste mitigation improving coastal ecosystem quality).Integrated aquaculture has many benefits, where bioremediation is one of the most relevant and yet unvalued in its real social and economic potential. Reducing risks is another advantage and profitable aspect of farming multiple species: a diversified product portfolio will increase the resilience of the operation, for instance when facing changing prices for one of the farmed species or the accidental catastrophic destruction of a crop

Species selection and seed availability

It is well known that availability of seed in adequate quantities is one of the major challenges in the development and expansion of mariculture. Though seed production technologies have been developed for many marine finfish and shellfish species, many of these technologies have not been scaled up to commercially viable levels. The hatchery seed production of many high value marine finfishes and shellfishes is complex and expensive due to the high costs involved in the establishment of broodstock and hatchery facilities and also to the complicated larviculture procedures involving culture of proper live feeds, their nutritional enrichment, feeding protocols, grading, water quality maintenance, nursery rearing and disease management. The production of seed of the concerned species by development of commercially viable technologies is essential for development of sustainable mariculture practices, but many of these technologies are still in the emerging state and may take several years for standardisation on a cost effective level.

Capture based aquaculture (CBA)

CBA is an alternative for those species for which hatchery technology is not developed. As hatchery technologies remain to be perfected for many species, fish farmers have to depend on 'seed' available from the wild. CBA has developed due

to the market demand for some high value species whose life cycles cannot currently be closed on a commercial scale. CBA is a world-wide aquaculture practice and has specific and peculiar characteristics for culture, depending on areas and species.

Nutrition and feed technologies

Currently, one of the most heated debates concerning aquaculture development is the use of fishmeal and other animal proteins in aquafeeds. Although fishmeal is used for its high quality protein content, it has several disadvantages, including high cost and instability of supply. Wild fish catches are on the decline and there are increasing environmental concerns (eutrophication, pollution associated with excess nutrient waste), ethical concerns over feeding fish to non-piscivorous fish, and social concerns over using aquatic protein to feed fish that could be used for human nutrition (especially in nutritionally deficient areas of the world) .Plant protein has significant potential for addressing the problem of phosphorus pollution, since plants do not contain the high levels of phosphorus found in animal protein. The use of plant protein in aquafeeds also helps reduce pressure on wild fish stocks. Research in this area is focusing on the investigation of various plant species and plant-animal protein mixes, as new sources for protein for aquafeeds for shrimp. Researchers are looking at the possibilities of dealing with anti-nutritional factors by producing feed enzymes to counteract them. Phytase is one example. This enzyme helps fish make optimal use of the phosphorous available in plant-protein based feeds.

Dependable availability of quality fry to stock grow-out production systems has been one of the most critical factors affecting commercial success of fish and shellfish production. Although nutritional and dietary requirements of most fish and shellfish species have been identified, large-scale hatchery production of most aquatic species still depends on live feeds, such as selected species of microalgae, the rotifer Brachionus and the brine shrimp Artemia. The live feed production systems used in most developing countries are still labour intensive. This lowers cost efficiency and poses many problems for consistent mass production, including optimal nutritional quality and prevention of microbial contamination. These problems have created a whole new area of biotechnological research aimed at finding cost-effective and efficient supplements to live microalgae, commercial production of freeze-dried algae, microencapsulated diets, and manipulated yeasts. Future aquaculture development ultimately depends on the ability of farmers and processors to produce a product acceptable to consumers. Increasing consumer demands for quality and safe products have to be recognised and addressed. Biotechnology also shows promise in this area, especially for assessing and improving safety, freshness, colour, flavour, texture, taste, nutritional characteristics, and shelf-life of cultured food products. Tools are already under development, or commercially available, that can detect and assay toxins, contaminants, and residues in aquatic products. Biotechnology tools can also be used to identify and characterize important aquatic germplasm resources, including those of endangered species. The genetic make-up of aquatic species can now be analysed, characterized and quantitative trait loci identified that code for phenotypic characters that are beneficial for culture (e.g., fast growth, disease resistance and cold tolerance). The study of biotechnology can also improve

understanding of gene regulation and expression, sex determination and definition of species, stocks, and populations.

Disease Management

Productions of specific pathogen free (SPF) and specific pathogen resistant (SPR) stocks are two complementary objectives being developed through shrimp broodstock management programmes. With establishment of mariculture of finfishes, research should be focused on these aspects in marine fish broodstock also. Taking this technology beyond specific pathogens, there is exciting potential for this approach to be adapted to selection of lines with high non-specific immunity or high tolerance of physiological stresses that facilitate opportunistic infections or other pathology. Considering the major contribution of many shrimp and finfish species to the global aquaculture production and the economic losses encountered due to both facultative and opportunistic disease outbreaks, it is appropriate and timely to concentrate further research to develop specific and non-specific resistant broodstock for commercially important finfishes and shell fishes.

Transboundary movements of aquatic animals have in some cases lead to the spread of aquatic animal diseases. Reliable and sensitive diagnostic techniques and standards are required to ensure such movements of live aquatic animals do not include the dispersion of their pathogens. Once DNA probes are field validated and refined for non-specialist use, these will be particularly valuable tools for this purpose.

One of the most urgent needs for aquaculture health management is establishment of standards for quantitative assessment of health status in the broad range of species under culture. Harnessing the host's specific and non-specific defense mechanisms in an effort to control aquatic animal diseases has considerable potential for reducing the impact and losses from diseases. Immunostimulants and non-specific immune-enhancers are being incorporated into diets to boost protection.

Probiotics are generally administered as live microbial feed supplements which affect the host animal by improving the intestinal microbial balance to optimise the presence of non-toxic species. A stable gut microflora helps the host resist pathogenic invasions, particularly via the gastrointestinal tract. Antibiotics reduce specific or broad-spectrum gut microflora and probiotics may have post-antibiotic treatment potential for restoring the microbial balance. Probiotics are widely used in animal husbandry but their use in aquaculture is still relatively new.

Bioremediation for environmental sustainability

Bioremediation is another promising biotechnological approach for degradation of hazardous waste to environmentally safe levels using aquatic microorganisms, or other filtering macro-organisms. In addition to microbes, bivalves, seaweeds, holothurians (sea cucumbers), etc., have been tested to assess their ability to reduce organic loading, or reduce excess nutrients produced during culture production. Various bioremediation preparations have also been developed with the view to remove nitrogenous and other organic waste in water and bottom sludge, to reduce chemically-induced physiological stress. Concomitant with bioremediation is enhanced feed delivery. Aquaculture development in recent years has, therefore, included investigation into methods for more efficient feeding.

Underwater closed circuit television is in use to record when fish are satiated (no longer feeding), so feeding can be halted, and also to monitor the accumulation of wastes under moored cages. Training fish to trigger feeding when hungry offers strong potential to lower feed costs, raise conversion efficiency and reduce wastage and pollution.

Conclusion

Establishing mariculture as a major sector needs lot of research and developmental intervention. Opportunities are immense in mariculture in India, and it has to be tapped in the most effective way. With proper funding support and technological advancements, mariculture can be counted for future as the sunrise sector in India.

Site selection for open sea cage mariculture- Advancements in India

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1. Introduction:

As the inland aquaculture has suffering with its own limitation in expansion due to resource constraints, pollution and other disease setbacks, the country's marine resources are seen as alternative for future development and expansion. To make use of untapped resource potential, country in need of sustainable indigenous farming systems which are technically adaptable and socially acceptable. Sea cage farming is a popular method of finfish rearing along the coast. This new technology requires fewer physical facilities, less space, a lower initial investment, and requires less capital to operate than multiday fishing.


Sea cages deployed along Somnath coast, Veraval, Gujarat

The significant development in sea cage farming in India occurred in May 2007, when the ICAR CMFRI began its first research and development of open sea cage trial in the Bay of Bengal off the Visakhapatnam coast. After the required modernization to the open sea cage culture technology, CMFRI created two versions of the circular open type sea cage, one with a 15-meter diameter and the other with a 6-meter diameter. Both versions were manufactured of High Density Polyethylene (HDPE) material. Later, open sea cages constructed of galvanised iron (GI) were created, and this is the most recent model of open sea cage which is now being used for efficient low-cost investment farming operations. (Philipose *et al.*, 2012).

Proper site selection for marine net cage culture is critical because it can significantly affect construction costs, operating costs, fish growth and survival rates, and the service life of the cages. Although floating cages can usually be towed away, it is always cost effective to do so. Site selection criteria also serve as a technical guideline for the creation of sea farming resource atlases, rules, and regulations, which are required for each country's mariculture development programme. Utmost care need to be taken while selecting site for cage as there is very less room for error. For example, in case of land based farming poor ponds can be improved by drilling boreholes to increase water supply, or by introducing filters. However, there is very little that can be done at the cage farm if the site proves to exposed and poor water exchange or if water quality deteriorate. Similarly, as the open sea cage farming is practiced in public spaces, there is very little control of sea cage owner over subsequent development, including establishment of other farms that may adversely impact the quality of site. Prior to establishing a cage culture system, a thorough understanding of the site's environment is required, which can be obtained through studies and analyses of field survey work, existing government data, available literature, and consultations with local people.

Fig. 1 Floating type HDPE cage deployed along Visakhapatnam coast

Even if a large portion of the data must be gathered by survey methods and the examination of water samples, communicating to locals about the current weather and the incidence of algal blooms or pollution can generate invaluable information. Prior to the construction of a cage farm, nearby residents should be consulted. This may assist reduce poaching and vandalism. In Indian region, stationary and floating type of cages are most common. The floating type has the advantage of being mobile and having greater potential in deeper waters. The stationary type, on the other hand, is ideal for small-scale farmers due to its low construction cost. Not all of the technical criteria for site selection, as expected, would apply equally to both forms. The guidelines discussed in this chapter are broad and general in nature, and they may need to be modified to suit local conditions and species to be cultured in each area. The criteria of site selection for sea cage farming can be broadly categorized into criteria of Physical, Chemical, Biological and

Topographical parameters. Additionally, it also pertains evaluation of Accessibility, Social issues and Legal aspect.

Fig. 2 Floating type GI sea cages ready to deploy along Somnath coast of Veraval

Fig. 3 Fixed type net cage
Source: FAO

Table 1. Important factors for Marine Finfish Netcage Culture Site Selection Criteria.

Physical Criteria		
Parameter		**Acceptable standard**
Water temperature or Sea Surface Temperature (SST)	27–31	°C
Current velocity	Min > 10	Max < 100 cm/sec

Turbidity/ Total Suspended Solid (TSS)	< 10	ppm	
Chemical criteria			
Dissolved Oxygen	>4	ppm	
Salinity	15-30	ppt	
pH	7.0-8.5		
Ammonia (NH_3)	<0.5	ppm	
Nitrate (NO_3)	<200	ppm	
Nitrite	<4	ppm	
Phosphate	<70	ppm	
Biological Oxygen Demand (BOD)	< 5	ppm	
Chemical Oxygen Demand (COD)	< 3	ppm	
Biological criteria			
Bacteria count (*E. coli*)		< 3000 cell/ml	
Topographical Criteria			
Depth	Stationary cage	min > 4, max < 8 m	
	Floating cage	min > 5, max < 20 m	
Wind velocity	Stationary cage	< 5 knots	
	Floating cage	< 10 knots	
Height of wave	Stationary cage	< 0.5 m	
	Floating cage	< 1.0 m	

(Ref. UNDP/FAO Regional Sea farming Development and Demonstration Project, RAS/86/024.)

2) Physical criteria:

a) Water temperature or Sea Surface Temperature (SST):

Cultured fish are typically exposed to seasonal fluctuations in water temperature during marine cage farming, and these changes fluctuate from location to location. A minimal increase in water temperature within the species' optimal tolerance limits stimulates growth but also reduces the dissolved oxygen concentration in water. Variation in the water temperature impacts fish activities and metabolism, oxygen intake, ammonia and carbon dioxide production, feeding rate, food conversion and ultimately growth. Water temperature normally changes with climatic conditions, but there exists a narrow range of variations in tropical regions compared to temperate zones. The yearly temperature range in the Asian region (tropical countries) ranges from 20 to 35°C and 2 to 29 °C in temperate countries. Solar radiation is however important for heat transport to the water column's top layers. Thermal stratification is possible in deeper, more or less stagnant bodies of water. Because low water velocity limits mixing of water during tides, it is observed that water temperature in shallow locations is often greater than normal. Three important factors which impact temperature fluctuation in coastal areas is land runoff, strong winds and currents. land runoff, brings colder water in winter/cold season and warmer water in

summer, winds and currents affects water temperature by bringing up the colder water from the bottom to the surface and reducing the heating up of surface waters. The optimum water temperature for cage farming purely depends on the cultured species. A temperature range of 27–31°C found optimal for most tropical species. As a result, it is recommended to choose fast growth species (not more than 8 months) and avoid prolonging the culture time into months with unsuitable temperatures.

Fig. 4 Multi parameter water testing kit (SST, pH, DO, Salinity etc.)

b) Current movement:

Tidal currents plays an important role in open sea cage farming as it brings oxygenated water to the cages removes waste matter from the sea cage. Strong currents will cause excessive strain on the anchoring system or fixed poles, distortion of the nets and cage structures whereas moderate water current speed will facilitate sufficient water exchange. Strong water current promotes lower growth in culture fishes as they have to spend most of their energy on swimming to maintain equilibrium and spreading food material outside the sea cage. Generally, large tidal range provides better conditions for high stocking density of fish in cages. It is therefore recommended to reduce the stocking density of fish, if there is strong water current in the coastal area. Additionally, an important consideration while placing a cage is the direction of the current. Circular cage design reduces the stress that strong currents put on the anchoring system and the cage frames. The maximum current velocity should be less than 50 cm/sec and not more than 100 cm/sec. If the highest current is less than 10 cm/sec, there will be insufficient water exchange, especially during neap tide, for intensive cage culture of fish.

c) Turbidity/ Total Suspended solid (TSS):

Water turbidity is an optical property of water where suspended and dissolved materials such as silt, clay, finely divided organic and inorganic matter, chemicals, plankton, and other microscopic organisms cause light to be scattered rather than

transmitted in straight lines. Total Suspended Solids (TSS) represents the actual measure of mineral and organic particles available in the water column. TSS is an important indicator of erosion and is linked to the movement of substances used in agriculture, industry, and transportation through river systems. Mostly silt and clay-sized particles make up suspended sediment, which can either be quickly moved downstream and locally deposited on floodplains or infiltrate into the bed's gravel interstices (Everest et al. 1987). Cage culture is not suitable in areas where turbid water exists which is typically caused by freshwater runoff during the rainy season. Soil erosion causes organic and inorganic materials to be suspended in the water column. Along with other industrial effluents, some heavy metals that were leached from the catchment region are also carried by run-off. The site's salinity can also decreased by the fresh water runoffs. Turbidity hinders the fish's ability to see their food, which can result in feed loss and slower fish growth. However, crustaceans like lobsters can withstand turbidity to some level without any negative impact on their ability to feed and grow. In addition, suspended sediments tend to clog fish gills which may lead to mortality from asphyxiation or proliferation and thickening of gill epithelial tissues which in turn affects the oxygen exchange capacity of gill filaments. The excessive occurrence of suspended solids also relates to disease such as “fin-rot” caused by Mycobacteria (Herbert and Merkens, 1961; Herbert and Richards, 1963). Suspended solids in a suitable site for cage farming should not exceed 10 ppm. But its effects also depend on the exposure time and current speed.

3) Chemical criteria:

a) Dissolved Oxygen (DO):

The DO requirement varies from species to species, pelagic fish like snapper and seabass requiring more DO than demersal species such as grouper. It is suggested that, the DO concentration should be preferably around 5 ppm or more and never less than 4 ppm for pelagic fish or 3 ppm for demersal species. In open sea cages, benthic organisms and sediment wastes may also reduce the oxygen level. Solubility of the water declines with increase in temperature and salinity; thus the sample of sea water contains less DO compared to equivalent volume of fresh water of the same temperature. While a sample of cold water contains more DO than the equivalent volume of warm water, provided salinity are same. As the altitude increases, there is decrease in partial pressure and a consequent reduction in the quantity of oxygen that the water can hold. The relationship is summarized in table 2. Due to these inverse relationship of water temperature and salinity with DO, rise in water temperature and salinity leads to depletion of DO level in sea cage, hence it is always notices depletion of DO during night time at neap tide in summer. Higher stocking density, limited water circulation due to excessive fouling, sediment waste in shallow water areas are some factor which also leads to depletion of DO in open sea cages.

Table 2. Solubility of oxygen in water (ppm) at different temperatures and salinities when exposed to water-saturated air at a total pressure of 760 mmHg (=1.01 bar) (from Sterling 1985 and Beveridge 2008)

Temperature (°C)	Salinity (‰)							
	0	5	10	15	20	25	30	35
0	14.6	14.1	13.6	13.2	12.7	12.3	11.9	11.5
2	13.8	13.3	12.9	12.5	12.1	11.6	11.3	10.9
4	13.1	12.7	12.2	11.8	11.5	11.1	10.7	10.3
6	12.5	12.1	11.6	11.3	10.9	10.5	10.2	9.8
8	11.8	11.5	11.1	10.7	10.4	10.1	9.7	9.4

10	11.3	10.9	10.6	10.2	9.9	9.6	9.3	9
12	10.8	10.5	10.1	9.8	9.5	9.2	8.9	8.6
14	10.3	10	9.7	9.4	9.1	8.8	8.6	8.2
16	9.9	9.6	9.3	9	8.7	8.5	8.2	7.9
18	9.5	9.2	8.9	8.6	8.4	8.1	7.9	7.6
20	9.1	8.8	8.6	8.3	8.1	7.8	7.6	7.3
22	8.7	8.6	8.3	8.1	7.9	7.7	7.5	7.2
24	8.4	8.3	8.1	7.8	7.6	7.4	7.1	6.9
26	8.1	8	7.7	7.5	7.3	7.1	6.8	6.6
28	7.8	7.7	7.5	7.3	7	6.8	6.6	6.4
30	7.6	7.4	7.2	7	6.8	6.6	6.4	6.1
32	7.3	7.2	7	6.9	6.6	6.3	6.1	5.9
34	7.1	7	6.9	6.7	6.4	6.2	6	5.8
36	6.9	6.8	6.7	6.5	6.2	6.1	5.9	5.7
38	6.7	6.6	6.5	6.4	6.1	5.9	5.7	5.6
40	6.5	6.5	6.3	6.2	6	5.7	5.6	5.5

b) Salinity:

Salinity is one of the environmental factors that influence the growth performance of many cultivable organisms in sea cages. Salinity controls osmotic pressure which in turn controls the ionic balance in fish. Selection of sites at the mouth of rivers which have a large catchment area should be avoided as there is a greater chances of salinity fluctuations due to rain water or agriculture or river run offs. This sudden reduction in water salinity, at these sites, as well as the long exposure to freshwater, may cause considerable mortality in many cultured fish. Most of tropical species can tolerate low salinities such as 10-15 ppt. Suitable sites for cage culture should thus have salinities ranging from 15 to 30 ppt, allowing farmed species to be altered in accordance with market demands. The following table 3 represents salinity tolerance range of some commercially important marine finfish species.

c) pH:

By definition, pH is the measure of the relative acidity and alkalinity of the water or any substance. The pH value of sea water lies in the range of 7.5 to 8.5 and the suitable pH for most marine species is from 7.0 to 8.5. Extreme values of pH can directly damage gill surfaces, leading to death and because it also affects the toxicity of several common pollutants (ammonia, cyanide), heavy metals (aluminium) (McDonald, 1983). The acid death point of finfish is around pH 4, whereas the alkaline death threshold is around pH 11. When the pH falls outside of the optimum range, fish growth slows, reproduction decreases, and disease susceptibility increases. Maximum incidences of pH fluctuation is mostly occurred in inland waterbodies such as lakes and reservoirs due to soil condition (alkaline or acidic soil), climate (geology of watershed area and limestone) acid rains (leads to reduced pH in fresh water bodies). Whereas, seawater pH mostly does not show marked seasonal or diurnal changes like fresh water. pH is not the major problem in marine sites, care must be taken in fresh water as there can be marked seasonal and diurnal changes.

d) Ammonia:

It is recommended that the ammonia-nitrogen level should be less than 0.5 ppm. The level of ammonia toxicity depends on the species of fish, water

temperature and pH. Ammonia is the major product of teleost excretion (Cheng et al. 2004; Tomasso 1994) and comprises the majority of nitrogenous waste in intensive aquaculture production. It is also the nitrogenous end-product of amino acid and protein oxidation. The rapid accumulation of ammonia (NH_3), and to a lesser degree nitrite (NO_2), can lead to mass mortality. Ammonia levels in water can be affected by the breakdown of uneaten food and detritus at the bottom. In most coastal areas, sewage discharge and industrial pollution are the primary sources of elevated ammonia levels in seawater. The suitable time for measurement of ammonia level should be during neap tide when water currents are considerably low.

Fig. 9 Ammonia, nitrite and nitrate testing

e) Nitrite (NO_2-N) and Nitrate (NO_3-N):

Nitrogen is generally the limiting nutrient in marine waters. Nitrite is a nitrogenous intermediate formed during the nitrification of ammonia by bacteria or through the denitrification of nitrate. Excessive amount of nitrite in water becomes toxic to fish due to oxidation of iron in haemoglobin from ferrous to ferric state (Tiensongrusmee, 1986). Since the haemoglobin molecule cannot interact with oxygen, it causes hypoxia in fish. Methaemoglobinemia is a disorder induced by both nitrite and nitrate that disrupts the normal structure of blood haemoglobin, transforming haeme groups into nitritebound forms. A methemoglobinemia caused due to Nitrite oxidation is more severe compared to nitrite oxidation hence the nitrite level in water body must be minimal whereas excess nitrate can be tolerated by finfish up to certain level. When dissolved oxygen levels under high density culture environments are disturbed, diminished oxygen binding ability of blood can result in fish suffocation and mass mortality. For a suitable farming area, nitrite level should not exceed 4 mg/litre while nitrate level should be below 200 mg/litre.

f) Phosphate:

Phosphorous is a limiting factor for productivity in coastal marine system. Increased level of Phosphate in coastal water may contribute to algal blooms and eutrophication (Cloern 2001, Nordvarg & Hakanson 2002). The phosphate content in natural coastal water ranges from 0.01 to 200 ppm, and an overabundance of phosphate in water will tigger over-blooming of phytoplankton, leading oxygen loss in cultured waters. A good cage culture site should have a phosphate level of no more than 70 mg/litre.

g) BOD and COD:

The main reason of rising BOD and COD demand in water body is the death of phytoplankton after blooming, uneaten feed, fish waste in the cage, sewage discharge and animal waste discharge, and industrial effluents. High organic load reduces the oxygen level in the water body, increase bacterial load which leads to the disease infection in culture fishes. The organic content in water is measured by Chemical Oxygen Demand (COD) which should be 3 ppm or less for a suitable site (Chou, 1988).

Besides organic matter in home sewage, other pollutants such as detergents and various hazardous compounds (cyanide, sulphide, chlorine, formaldehydes, phenols, oil, and so on) have an impact on cage farming. Other than animal waste, agricultural wastes such as pesticides and herbicides frequently leak into the culture site and can accumulate in fish or kill them. The normal measure of the degree of pollution is the Biological Oxygen Demand (BOD) which should not exceed 5 ppm at 5 days period (Tiensongrusmee, 1986).

h) Heavy metal toxicity:

Pollution in coastal water bodies not only leads to eutrophication but also leads to excessive heavy metal deposition in the bottom sediments and aquatic organisms. These heavy metals get deposited in the farmed fishes which is ultimately harmful for human beings once they reached beyond threshold limit. Hence it is important that the site of cage culture should be as far as away from the industrial areas or effluent discharge areas. Below mentioned table 4 highlights acceptable limit of heavy metals for site selection of sea cage farming.

Table 4. Acceptable concentration of heavy metals for sea cage site

Heavy metals	**Acceptable concentration (ppm)**
Mercury (Hg)	<0.004
Cadmium (Cd)	<0.03
Copper (Cu)	<0.01
Aluminium (Al)	<0.1
Zinc (Zn)	<0.1
Lead (Pb)	<0.1
Chromium (Cr)	<1.0
Iron (Fe)	<1.0
Manganese (Mn)	<1.0
Tin (Sn)	<1.0

4) Biological criteria:

Domestic, industrial or agricultural sewages is the main source of most pathogenic or potentially pathogenic organisms spread to the cage farms. The 'red-boil disease' in estuarine grouper (*Ephinephelus salmoides*) is produced by the bacterium, *Vibrio parahaemolyticus* which is commonly found in sewage-polluted culture waters (Wong et al., 1979). The amount of *E. coli* in water is used as an indicator to estimate the level of recent pollution as well as the possibility of diseases infection in fish and humans. A good cage culture site should have an *E. coli* count of less than 3,000 cells/ml. The main sources for disease in open sea cage farm can be predators which include sea birds, puffer fish and crabs can also carry diseases and some small fishes from the surrounding cage environment, also aggregation of a large number of cage farming units in the same area will cause the outbreak of diseases, especially when the cages are in existence for a longer period of time.

Other biological factors includes Harmful Algal Blooms (HABs), Plankton and fouling organisms. Fish may die as a direct result of poisoning, gill damage, or growth and vigour loss when HABs occur near fish farms. Dinoflagellates are the most important type of toxin-producing algae (causes red tides). Excessive phytoplankton blooms will occur if proper conditions, such as high light intensity, high nutrient levels, high water temperature, stagnant hydrological conditions, and so on, predominate. Algal blooms can endanger fish not just by blocking their gills, but also by competing for dissolved oxygen at night. Blooms are formed by a variety of marine algae taxa, including diatoms, Cyanobacteria, prymnesiophytes, and dinoflagellates. *Chaetoceros convolutus* a diatom species, contains numerous conspicuous spines that interfere with gill function and cause blood loss through injuries. Fouling organism mainly causes clogging of net in sea cages and leads to restricting water flow limiting dissolved oxygen and waste elimination in the net cages. The silt particle accumulation over the cage nets is the primary initiator of fouling organism colonisation. Depending on the region and season, silt particles can account for more than half of overall fouling weight (Chou, 1988). If excessive fouling occurred due to barnacles, bivalves, algae, etc. will lead to the increase in the total weight of the cage nets and thereby the difficulty in exchange and cleaning of the culture nets.

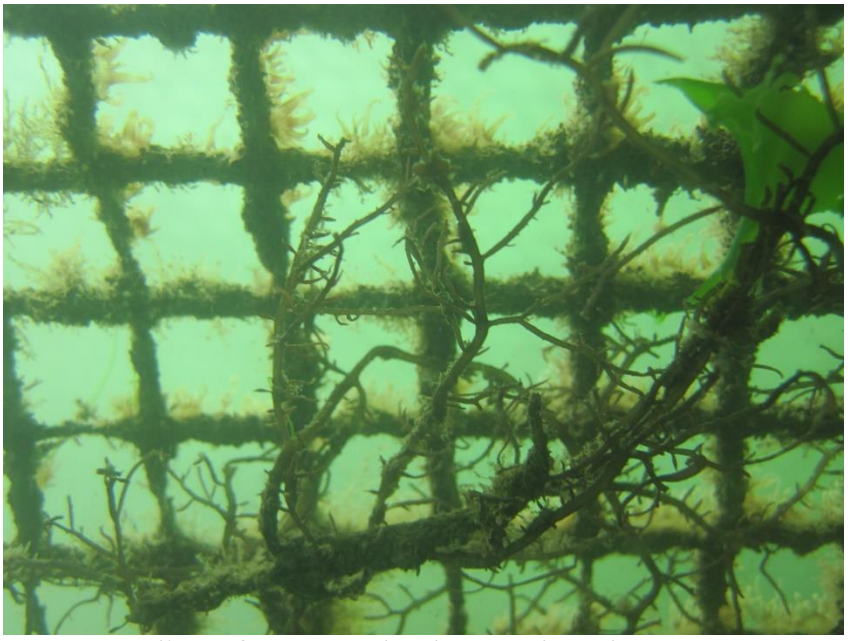

Fig. 10 Fouling of cage net leads to reduced water movement

Fig. 11 Fouling of the cage structure

5) Topographical criteria

a) Depth:

The cage location should be suitable in depth, have good tidal flow with optimal conditions, and ideally be protected from strong winds and bad weather, with sufficient water flow. Since the typical depth of a cage is 2-6 m, sufficient depth under the cage is required to maximise water exchange, avoid oxygen depletion, the accumulation of uneaten feed, faeces, and debris, disease infection, and the build-up of some noxious gases such as H_2S produced by the decomposition of the deposited wastes. At the lowest low level of spring tide, the clearance for a floating cage should be at least 2-3 m. However, a stationary cage is allowed 1-2 m of clearance to reduce the expense of fixed poles. Furthermore, fixed cages are typically installed near the mouths of rivers, creeks, and canals where the water flow is greater than in the open sea. On the other side, the maximum depth of the floating cage should ideally be less than 20 m, as longer anchoring lines and heavier anchor blocks will be necessary, increasing investment and maintenance expenses. Since it is difficult to find adequately strong supporting posts longer than 8 m, the maximum depth of a stationary cage should not exceed 8 m.

b) Wind velocity and Height of waves:

Strong winds such as those generated by a cyclone will destroy any structure projecting above the water hence it is suggested that near shore cages should be sited in sheltered areas protected from strong wind. Generally, the wind velocity should not exceed 5 knots for stationary cage and 10 knots for floating cage. In relation to the wind speed, the height of the wave in a suitable area should preferably not exceed 0.5 m for stationary cage and 1.0 m for floating cage. Waves are also created from the wake of passing vessels, hence culture site should be at some distance away from the navigation routes.

6) Accessibility:

Good accessibility aids in the distribution of farm products (particularly live fish), as well as the transportation of feed, fingerlings, fuel, farm equipment, supplies, and other essentials. The culture site should be close to a coast, preferably with a jetty for boat connections to farms, and close to a good road for land transportation. The supervision of the farm by the concerned is able to ensure proper management if it is easily accessible. In most of the large scale-intensive cage farms, there are boarding and lodging facilities on floating rafts or on the shore close to the cages which majorly includes an office, feed store, laboratory and dormitory.

7) Social issues:

Security is a most important consideration for all types of marine cage farms. Since the cage culture are sited in public waters, it is an easy target for the people bent on theft or vandalism. Some countries in the region have legal rules and regulations in place to protect cage farmers' products through insurance. As a result, farmers must be extremely cautious to prevent poaching, or choose a site far away from such potential areas. These will also raise production expenses in terms of security, transportation, and management. In Thailand, the owner practice setting up of sea cage near to their home, but it brings in problems related to sewage discharge from village.

Fig. 12 Local villagers ('*Sidi*' Adivasi tribe) and community head involved in sea cage activities

Various large scale farmers face conflict with the local villagers especially when they may have to hire the killed or cheaper labour from outside the village. Such type of conflicts always leads to undesirable results finally it will lead to poaching problem or the destruction of the cage farms. In order to avoid such type of conflicts, it is good to take leader of the village into confidence. Other common users of the sea may cause conflicts, such as waves or oil leaks from boats, pollution from industry, garbage from other farms, and oil spilled from tankers or shipyards. Fish husbandry in net cages should not impede other stakeholders of

the water resource, such as those engaged in fishing, boating, leisure activities, navigational channels, ports, harbours, tourism, and so on. According to the guidelines of the Government of India's National Fisheries Development Board (NFDB), sites that are active fishing zones and close to harbors/fish landing centres and navigation channels, defence areas, marine protected areas, coral reefs, mangroves, areas under coastal management plan, points of industrial effluent discharge, sewage pollution, heavy freshwater discharge by rivers, presence of underwater pipelines, telecom cables, explosives dumping, areas of historic ship wreck all prohibited for site selection.

8) Legal Criteria:

Since fisheries are a state subject under Article 21 of the Indian Constitution, management and control of fisheries up to territorial waters is placed in the governments of the states and union territories. As per the Article 21 of Indian Constitution, states have the authority to regulate and manage marine fisheries in their territorial waters that extend up to 12 nautical miles off the shore into the sea. Since 1980, all maritime states have passed Marine Fisheries Regulation Acts. The Union Government has control over the EEZ from 12 nautical miles to 200 nautical miles. The clauses of the 73rd and 74th amendments to the Indian Constitution authorise Panchayats to fulfil the functions listed in the eleventh schedule of the Constitution 29 subjects including fisheries. However, due to lack of legal clarity this has not been implemented in any Panchayat.

Fig. 13 Sea cage installation

Most of the countries in the Asia and southeast Asian region have a standard law on lease of public water for any construction and for fisheries. In some

countries in the region cage farmers have to obtain license to culture fish in cages from the respective government bodies with restrictions concerning site, species, size, structure, etc. The competent government should identify the site for cage culture in a scientific way so as to avoid competition with the other common users of the water area and interference with local navigation regulations. In Indian context, it is recommended that the layout plan and strength of cage structures should be approved by the government and/or scientific organizations. The existing regulations must be carefully studied to avoid any obstacles, and that lease and license (if any) should be applied for as soon as possible due to the lengthy processing involved in obtaining permission due to the involvement of many government departments for the same. The concerned government entities must provide adequate enforcement facilities for the enterprise to develop indefinitely.

9) Case study:

Suitable site selection for open sea cage farming via traditional method is time consuming, labour intensive, and exorbitant process. The utilization of cutting-edge Geographical Information System (GIS) and remote sensing (RS) technology allows users to locate the best suitable areas for marine cage farming, by saving time and money. Through the use of satellites, RS technology enables users to collect geographic and environmental data about a specific location or area, and GIS enables users to analyze these data to map out potential sites in the area. The clearest illustration of the application of new satellite technologies in the selection of mariculture sites is a recent research by the ICAR CMFRI along the Gujarat coast.

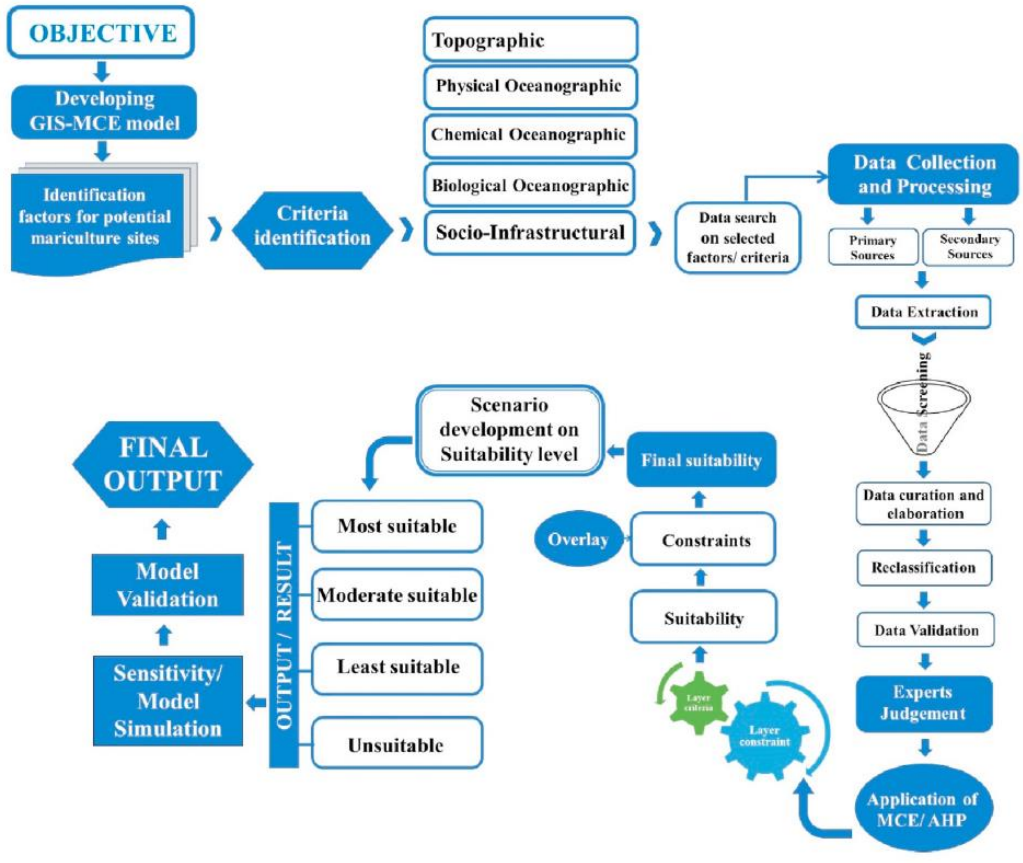

Fig.14 Conceptual schema designed to develop the decision-making spatial model.

A preliminary Geographic Information System (GIS)-based decision support model and spatial framework for site selection of cage farms in Gujarat state, India's territorial waters around the Arabian Sea were developed. This analysis evaluated a trajectory of 20 kilometre sea space covering 23,949.33 km^2.

In addition to the preferred biological and oceanographic collections for the culture system and candidate marine fish species, data on maritime aids, sewage, industrial outlets, river mouths, ecologically sensitive locations, and other constraints were marked, re-classified, optimised, and benchmarked for decision-making analysis. Other sub-models considered in the proposed comprehensive model include topographic, physical, chemical, and biological oceanography models, as well as socio-infrastructural models.

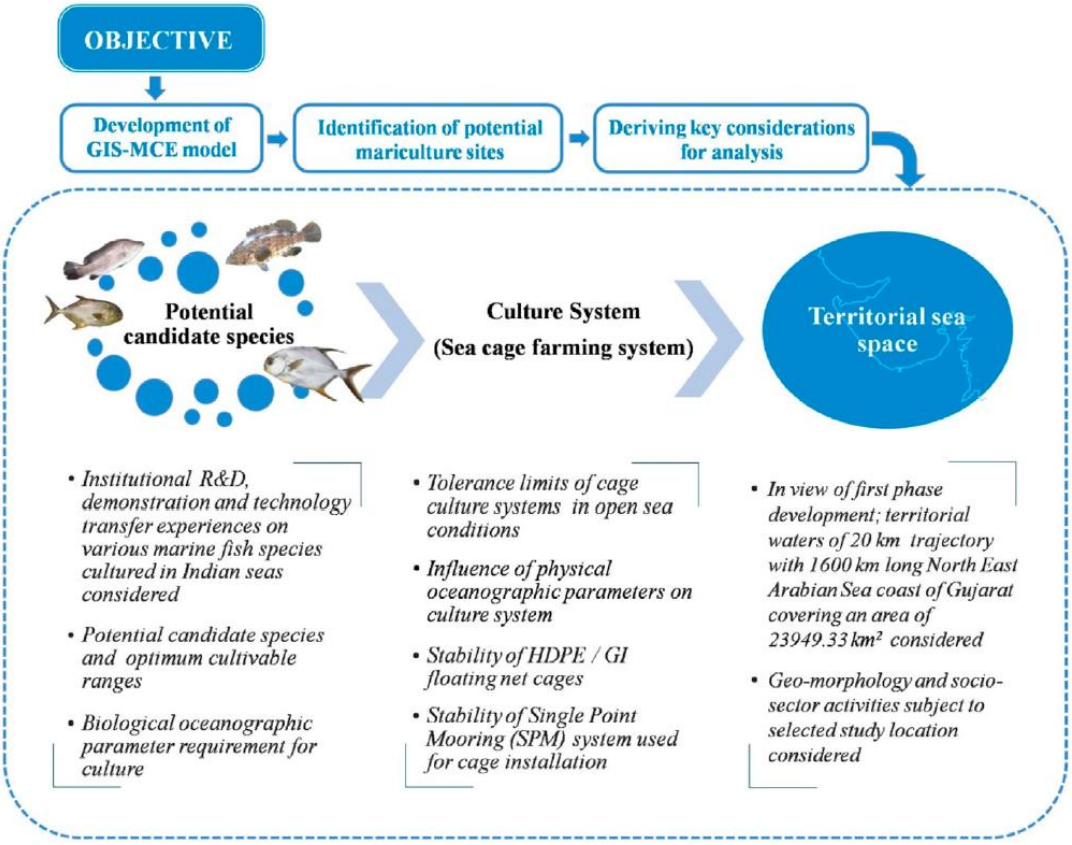

Fig. 15 Schematic representation of factor consideration to locate potential mariculture sites along North East Arabian Sea coast of Gujarat, India.

The model explored and delimited 12,557.74 km2 (52.43% of total) appropriate maritime space for mariculture. Out of the demarcated area, 27.43% was most suitable and 25.00% was moderately suitable for mariculture development, highlighting the untapped potential of Gujarat state's abundant open seas. According to the sensitivity simulation, the proposed systematic analytical GIS-Multi Criteria Evaluation (MCE) model was successful, stable, and provided an efficient solution for complex spatial issues in the mariculture site selection process.

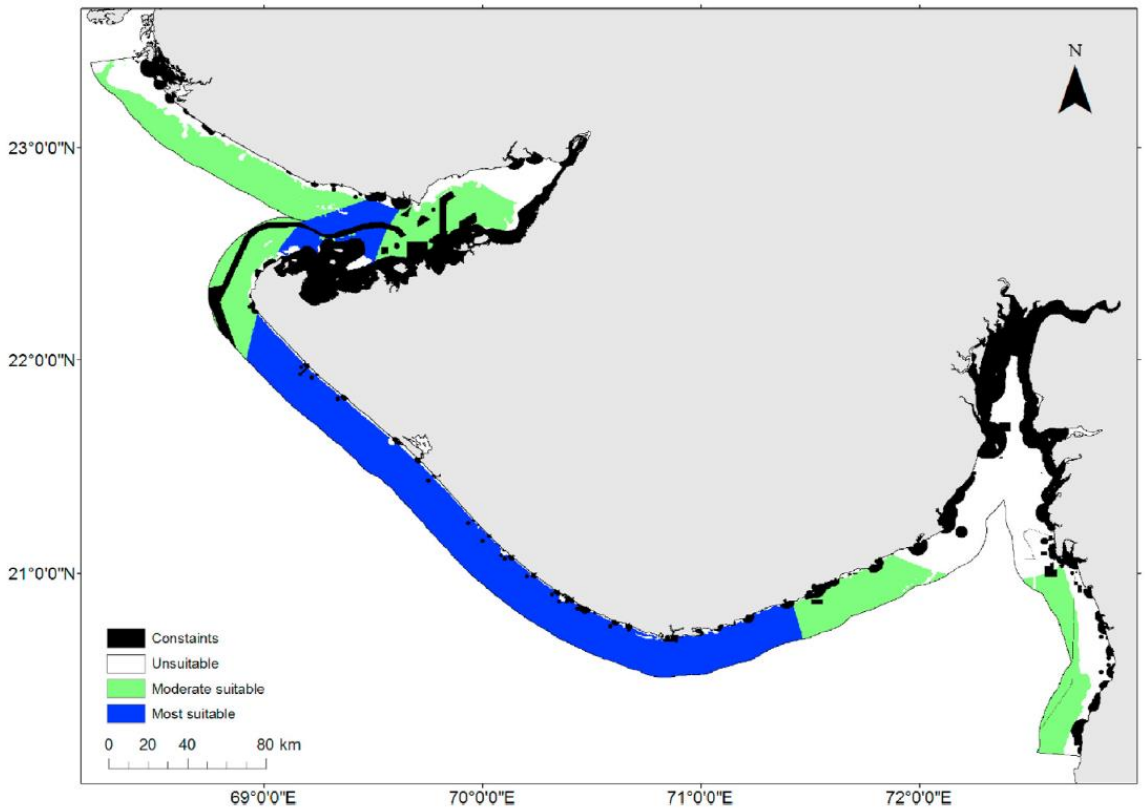

Fig. 16 Final suitability map for potential sites for mariculture development

Furthermore, these findings revealed that the current spatial decision support model, specifically its methodology and structure, enabled the identification of the best suited areas for mariculture along the country's territorial seas. The model was transferable to all of this subcontinent's marine nations and might be an effective and valuable tool for resolving the complicated spatial challenges involved with the site selection process for mariculture in open seas (Divu *et al.,* 2020).

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Sea Cage Farming

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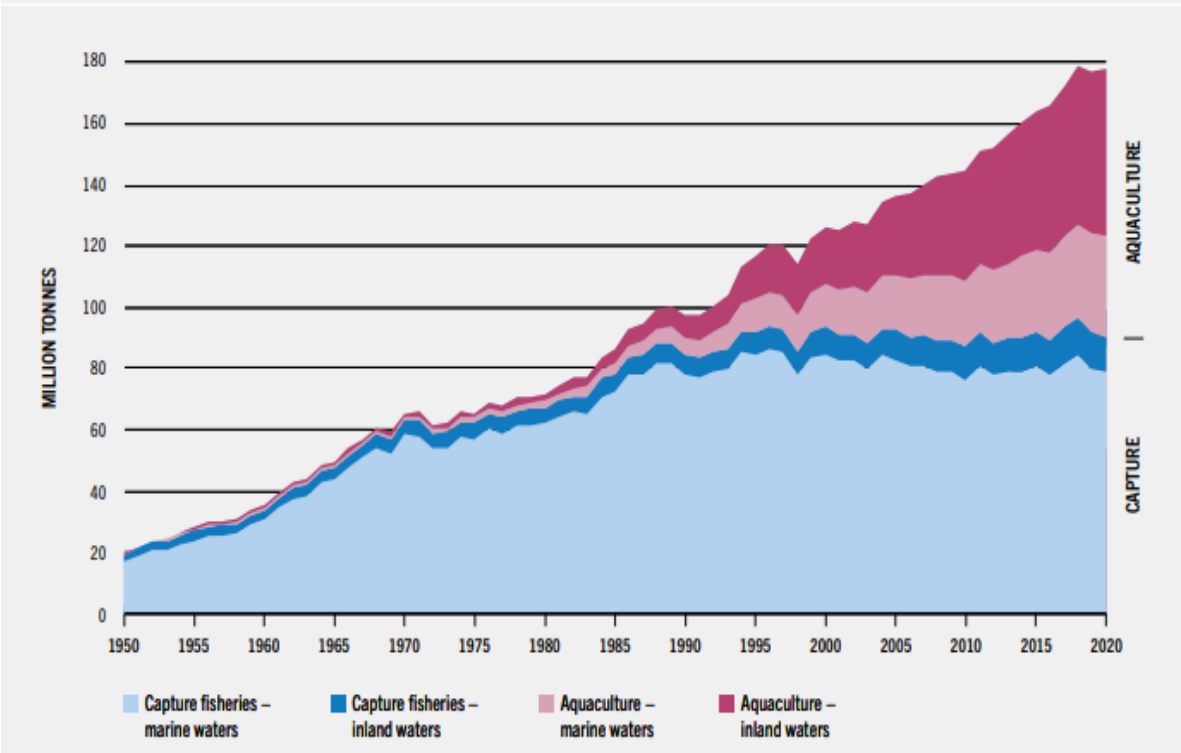
Globally, aquatic foods provide about 17% of animal protein, extending over 50 percent in several countries in Asia and Africa. The sector employs an estimated 58.5 million people in primary production alone, approximately 21 percent women. In 2020, total fisheries and aquaculture production worldwide reached 214 million tonnes (mt) worth about USD 424 billion. Out of the total production, 90.3 mt accounted from capture fisheries (2% drop

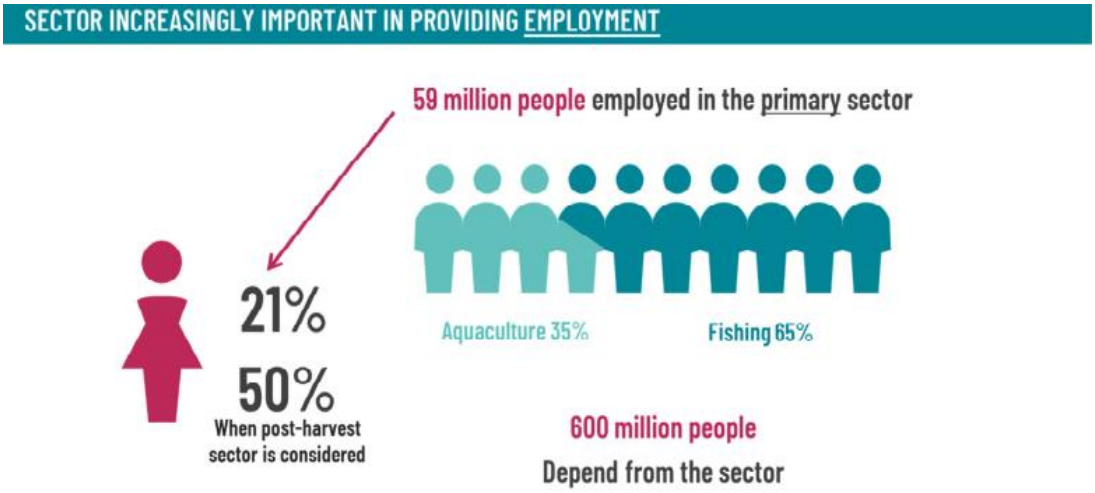
from last year), 87.5 mt was contributed by aquaculture and 36 mt was from algae



production (FAO, 2022). Production of aquatic animals in 2020 was more than 60 percent higher than the average in the 1990s, considerably outpacing world population growth, largely due to increasing aquaculture production. In 2020, 89 percent (157 million tonnes) of world production (excluding algae) was used for direct human consumption. On a per capita basis, consumption of aquatic food grew from an average of 9.9 kg in the 1960s to 20.2 kg in 2020.

Global capture fisheries production (excluding algae) represented a fall of 4.0 percent compared with the average of the previous three years. On the contrary, aquaculture sector represented a 2.7% growth from the previous year. Global aquaculture production in 2020 reached a record 122.6 million tonnes, including 87.5 million tonnes of aquatic animals worth USD 264.8 billion and 35.1 million tonnes of algae worth USD 16.5 billion. Around 54.4 million tonnes were farmed in

inland waters and 68.1 million tonnes came from marine and coastal aquaculture. Currently we farm about 652 species in aquaculture sector. Aquaculture sector continued to grow in all regions of world, except Africa in 2020. The aquaculture sector remains the fastest growing food production system in the world. Asia continued to dominate world aquaculture, producing over 90 percent of the total. With the dwindling production from capture fisheries sector, aquaculture sector should be focused and developed to meet the food security and nutrition.



Aquaculture is the controlled cultivation of fish, crustaceans, mollusks, algae and other organisms in freshwater, brackish water and saltwater. Fish can be cultured in any of the four culture systems such as ponds, tanks, raceways, recirculatory systems or cages. With the growing population and demand of fish, it is projected that over 60% of seafood must be contributed from aquaculture (mariculture) sector by 2030. Mariculture refers to the cultivation of marine organisms in seawater, usually in sheltered coastal or offshore waters. Mariculture may consist of raising the organisms in or on artificial enclosures such in floating netted enclosures (cages) for fishes or on racks for mussels/oysters or raft for seaweeds. Mariculture offers opportunity and has great potential to meet the fast-growing demand for seafood in India. Pioneering efforts to develop aquaculture in the country was started in the 1970s by ICAR-Central Marine Fisheries Research Institute (CMFRI) in Mandapam and Tuticorin with seaweed and bivalve cultivation. Since then, ICAR- CMFRI is in the forefront towards development and disseminating mariculture technologies in India through standardising seed production technology and farming of marine finfishes/shellfishes, Integrated Multitrophic Aquaculture (IMTA), Recirculating Aquaculture System (RAS).

Sea Cage Farming

Cage culture involves growing fishes in the sea while being enclosed in a net cage which allows free flow of water. It is a production system comprising of a floating frame of varying dimensions and shape, net materials and mooring system, to hold and culture a large number of fishes. In order to make the cage aquaculture economically feasible, it is essential to select proper design, ideal construction material, techniques, suitable mooring and good management practices. Sea cage culture is receiving more attention in the recent years both by researchers and

fishermen. Many small or limited resource farmers are looking for alternatives to traditional fishing practices. Cage aquaculture appears to be a rapidly expanding and it offer opportunities even on a small scale.

Understanding the importance of cage culture, the Central Marine Fisheries Research Institute has initiated cage culture as a research and development activity to identify appropriate design and suitability of cages under Indian context in the year 2006-07. The first open sea cage was launched in Bay of Bengal off Visakhapatnam coast during May 2007. This was a indigenously designed and fabricated 15 m HDPE cage provided with a cat walk for free working on board and stabilization. After modifications to the design, first successful harvest was made in 2008 from an HDPE cage of 15 m diameter which was stocked with seabass during December 2007. This cage which was stocked with sea bass was harvested after 6 months with 75% survival. After several trials and refinements, 6 m diameter cages were designed for ease of operation and economic point of view. Recently it has been projected by ICAR-CMFRI that even if 1% of the inshore waters is used for cage farming, which can have room for 8,20,000 cages with a production potential of 3.2 mt. ICAR-CMFRI is regularly involved in promoting open sea cage culture in all the maritime states through various demonstrations.

Advantages of Sea Cage Farming

Farming fish in ponds is by far the most widespread technique used, but fish farming in cages is gradually becoming more and more popular. Following are the advantages of open sea cage farming over other farming practices

- Fabrication of cages for fish farming is faster, simpler, and cost effective than onshore farms.
- The initial start-up investment required to produce one unit of fish meat is only 30 to 40% of the investment needed for conventional pond aquaculture.
- Cage aquaculture utilizes natural bodies of water, so it does not take up valuable space on land that can be used for other purposes.
- The location and size of cages can easily be changed, so the aquaculture operation can expand more easily.
- Unutilized areas such as open sea, lagoons or sheltered bays can be utilised for farming of fishes in net cages with prior permission from concerned agencies
- It is easier and quicker to harvest fish in cages.
- Farming fish in cages makes it easier to ensure a steady supply to meet market demands.
- Alternate livelihood and additional income

Types of cages

Several different types of cage designs are used for farming the fish. The suitability of each cage design depends on the aquaculture site – the water depth, flow conditions, and the environmental setting (river, canal, lake, reservoir, sea). The species of fish being farmed is also a key consideration, as various species have different feeding habits, general behavior, and stocking densities. Based on installation, cages are categorized into following types.

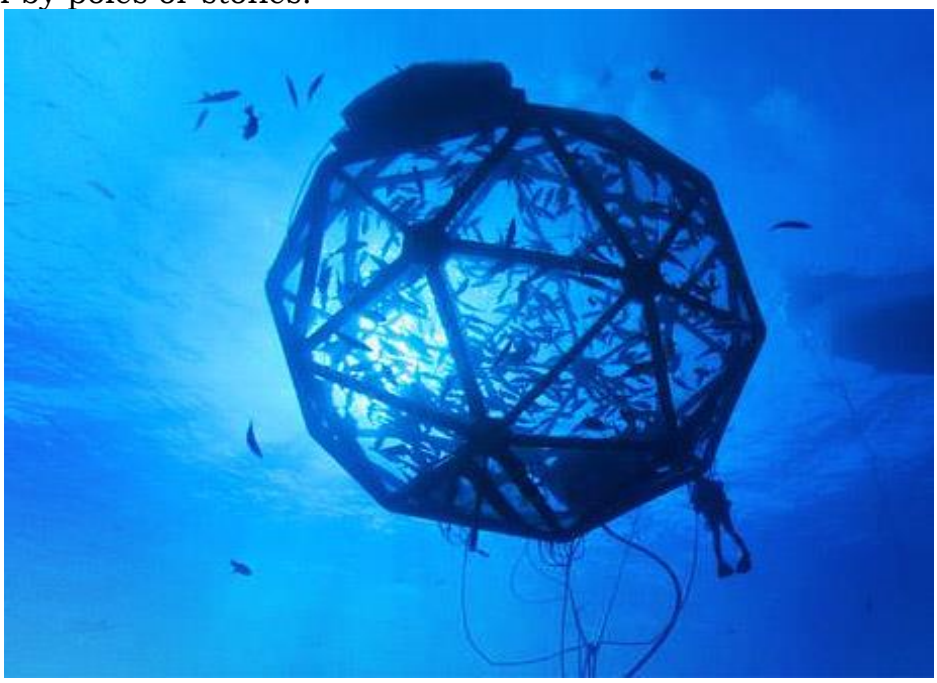
1. **Fixed**- Fixed cages consist of a net bag suspended from posts in the flow of a stream, river, canal, lake, or reservoir. These cages are generally used in shallow water bodies, with depths less than 3 meter. This type of cage is very basic and

low-cost to fabricate. Fixed cages are commonly used in small-scale aquaculture, but their use for more extensive, commercial aquaculture is restricted.

2. **Floating**- these type of cages are designed with the buoyant collar supporting the net. These cages can be made in variety of designs like square, rectangular or circular to suit the purpose of the farmer and are widely used. Rigid materials such as GI pipes, bamboos and plastic pipes can be used as frames. The floating unit consists of a number of floats below the framework to provide sufficient floatation. The types of floats used vary from ordinary oil drums to used fibreglass barrels. These cages are generally used in water bodies with depths ranging from 10-15 meters.

3. **Submersible-** Submersible cages are a variation of floating cages with either rigid or flexible netting. The buoyancy of plastic floats is variable so that the fish cage can be moved to different depths in the water column. These types of fish cages are often used in marine environments.

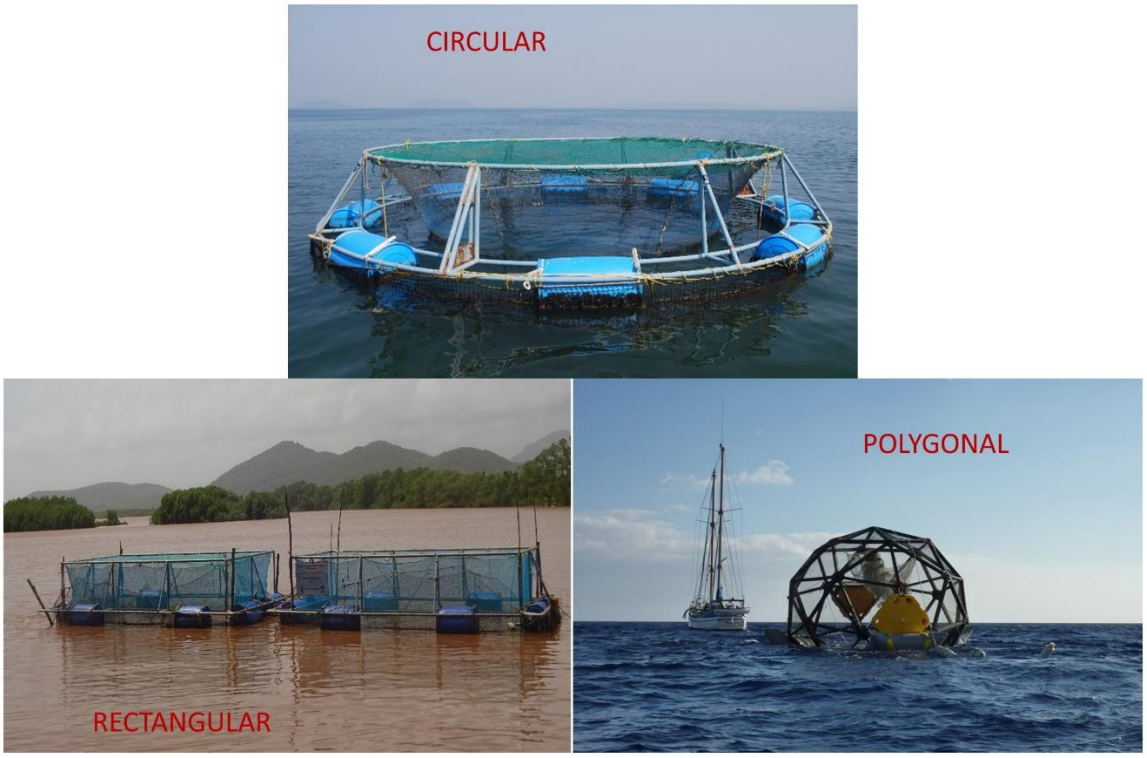
4. **Submerged**- these types of cages are made with wooden boxes having gaps between the slots to facilitate the flow of water and are anchored to the bottom of the substratum by poles or stones.



Cage design

Design of cages is a critical factor that pushes the limits of structural integrity and economic viability in aquaculture system. Design of cage is determined by the behaviour of the cultured species. For pelagic species which swim near the surface, bigger net space is required. Such fishes tend to aggregate in shoal and swim around in circular motion. Therefore, circular or hexagonal cages may be more suitable than rectangular or square cages Whereas, demersal fishes, which are less active, territorial inhabit and prefer to hide with underwater structure, the shape of the cages does not affect fish mobility. Under such

circumstances, square or rectangular cages have an advantage over a circular or hexagonal one in view of easy assemblage of cages and management. From the economic point of view, the design should be technically simple, should be easily made with available materials, cost effective, should hold reasonable amount of water while permitting sufficient water exchange and hold the fish securely during the culture period. . A good design must be safe, secure and easy to operate. Design of the cage and its accessories can be tailor-made in accordance to the individual farmer's requirements.



Different types of cage designs

Size

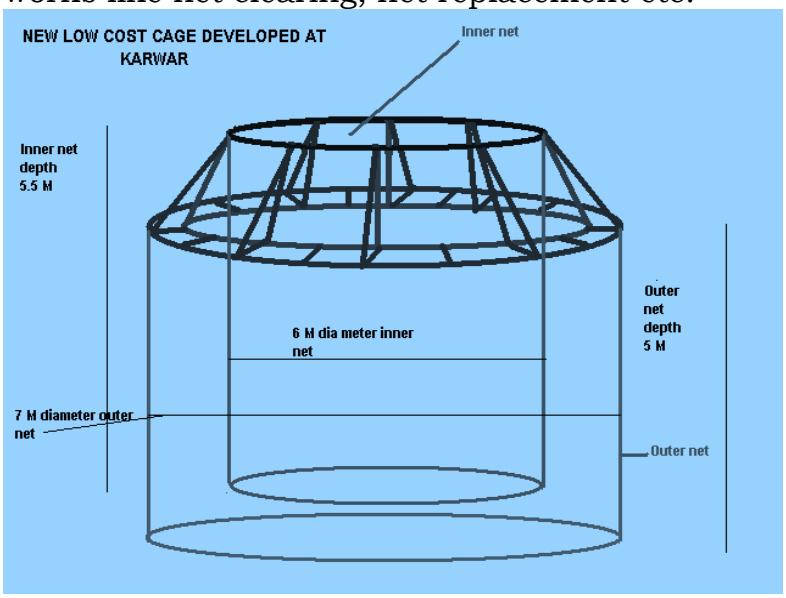
Size of the cage depends on the site, species used for culture and materials used for construction. It is a fact that cost per unit volume decrease with increasing cage size, within the limits of the materials and construction methods used. It is not economical to have a cage size beyond the physical capability of the fish farmers to handle. In tropical waters, the net could get fouled in a relatively short period of time and the weight of the net would be considerably increased rendering cleaning of the net difficult. This will also reduce the floatation of the framework on which the net are suspended. Size of the cages used in marine water is larger in size than freshwater. CMFRI has developed open sea cages of 6 m dia, 10m dia, 12 m dia and 15 m dia for grow out fish culture. Ideal size for grow out cage is 6 m due to its easy maneuvering and reduced labour.

Cage frames

Commonly used materials for marine cage frames are mild steel (MS), galvanized iron (GI), poly-vinyl chloride (PVC) and virgin-grade HDPE (High Density 5 Polyethylene). Frames of iron and steel (unless galvanized) should be coated with a water-resistant substance like epoxy, or an asphalt based or swimming pool

paint. In India, HDPE PE100 and GI pipes B/C class are preferred for cage frames. Cages frames are preferably made circular since circular cages can withstand sea conditions better than rectangular or square shapes. Business entrepreneurs with high capital investments can use long lasting and expensive HDPE frames can be used. Small groups and fishermen can opt for cost effective epoxy coated Galvanized Iron (GI) frames, which can be used for 2-3 years with proper maintenance.

The low-cost cage developed at Karwar is made of good quality 1.5" GI pipe (B class). The diameter of the cage is 6 m and the height is 120 cm from base to the railings. All the joints are double welded for ensuring extra strength. After fabrication the structure was provided with single coat epoxy primer and double coat epoxy grey paint to prevent rusting. The total weight of the cage is about 700 kg. The cage frame is kept floated in water by using 8-10 fibre barrels of 200 l capacity filled with 30 lb air are used for floating the cage. The cage when floated on inflated barrels provides a stable platform around the cage where fisherman can stand and safely carry out works like net clearing, net replacement etc.



Low cost cage developed by CMFRI (6m dia)

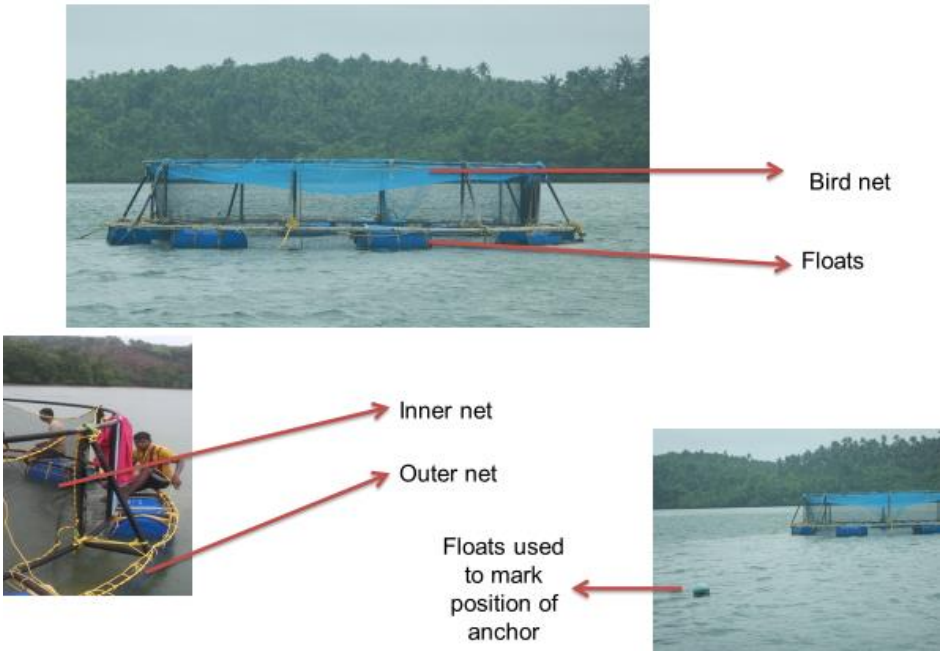
Net cage

Net cages are designed as per the shape and size of the cage and depth of the site. Synthetic twines manufactured from HDPE are totally resistant to sea water, acids, alkalies and chemicals. They do not absorb water and cannot rot very easily. HDPE is easy for handling and cleaning and are used for fabricating net cages for open sea cage farming. Also, nets made out of HDPE can last for two or more seasons with proper maintenance. The mesh size of the nets should be selected according to species and ensuring proper water exchange.

Dimensions of mesh size of net cages used for rearing/growout

Species	18 mm Mesh Fish Size (mm/g)	25 mm Mesh Fish Size (mm/g)	40 mm Mesh Fish Size (mm/g)	60 mm Mesh Fish Size (cm/kg)
Cobia	100-200/ 10-70	200-450/ 70-1100	460-750/ 1100-4000	75-100/ 4-7
Pompano	20-30/ 2	40-100/ 35	100-200/ 500	--
Seabass	20-100/ 15	40-200/ 300	200-400/ 1500	--
Grouper	20-100/ 15	40-200/ 300	300-400/ 1000	--

For sea cage farming, 3 types of nets are essential:
(i) Outer Predator Net
It is essential to prevent entry of predators in sea cage culture. Considering the strength, durability and cost factor, usually braided UV treated HDPE netting of 3 mm thickness and 80 mm
mesh size is found very effective and recommended. Dimensions of predator net cage – 7 m diameter and 5-6 m depth.
(ii) Inner Net
It is the net in which the seeds of desired species of fishes are stocked and cultured. For fabrication of inner fish rearing/grow-out net cage, twisted HDPE netting of 0.75-1.5 mm thickness and 12 - 40 mm mesh size is selected depending on the size of cultivable species. Dimensions of fish rearing net cage – 6 m diameter and 5-6 m depth
(iii) Bird Net
To prevent predatory birds from preying on fish, a protective bird net must be overlaid on the cage frame. HDPE or nylon nets of 60 - 80 mm mesh size will be ideal for a bird net.



The ballast pipe is another support system required to maintain the shape and structure of the net bags. Normally 1.5 to 2 inch diameter HDPE ballast pipe with holes at regular intervals, for the free flow of water, is used. Metal lines are inserted inside the pipe for increasing weight so that the ballast remains submerged in water

Mooring

Mooring system is used to hold the cage frame in a suitable position according to the prevailing environmental conditions. A good morring system is required to keep the cages in a fixed position and to reduce the transfer of excessive forces generated by wind, currents and waves to cages. In well protected bays and seawater sites and freshwater sites, the forces of exerted by environmental factors are less and thus, small mooring system can used. In the case of sea cages, where the cages are exposed to greater environmental forces require more effective mooring systems. Mooring joints the cage with the anchor system. Type of mooring

system to be used depends on the type of cage, site where the culture practices will be done, and the requirement for positional precision. Cage and mooring design is "site specific", and careful and combined choice of cage type, nets and most specifically moorings, has a considerable bearing on the ability of fish stocks to survive in major storms, on exposed sites. Good mooring system must be

- be strong enough to resist the forces of
- Currents
- Waves and Wind action
- It should withstand and transmit the forces acting on it.
- mooring line must have high breaking strength

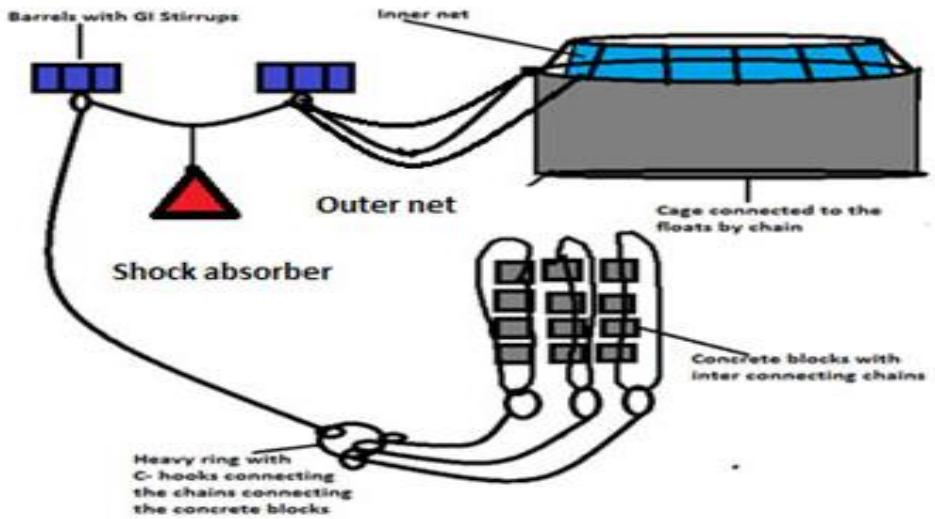
Mooring components

Important components include the anchor or mooring unit on the seabed, the rising line, which connects the anchor to the surface system, and the surface or subsurface mooring grid. The major elements comprise several smaller sub-units – particularly links, shackles, droppers, safety lines, buoys, etc., which in effect are integral in the complete system.

Types of mooring

- Single point
- Multipoint

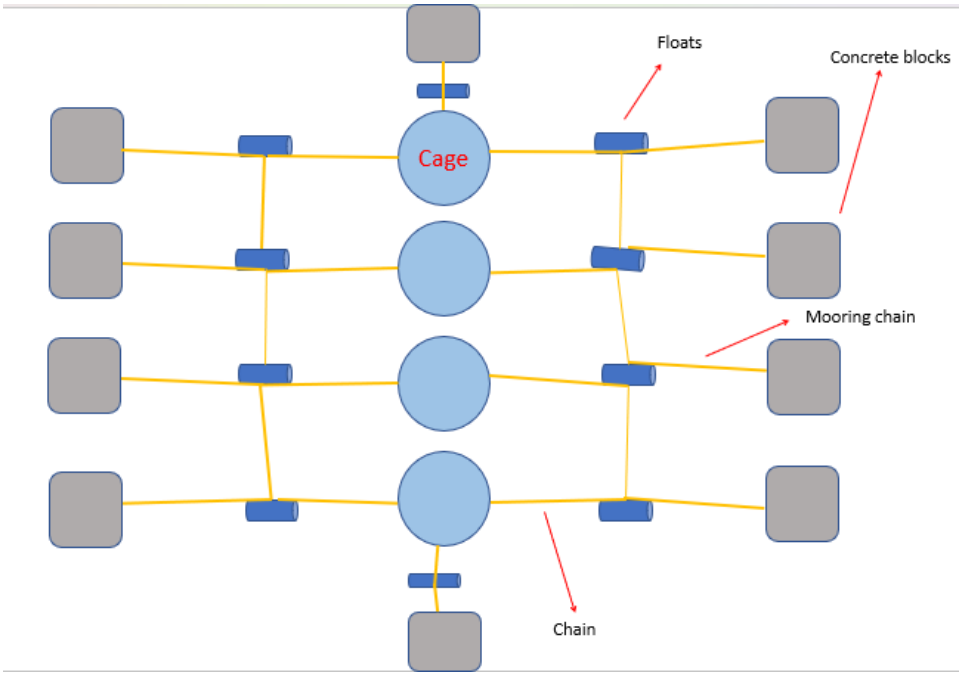
Single point mooring is used with rigid collars/ frames and this system allows the cage move in a complete circle. They use less cable and chain than multiple point mooring. Reduces the net deformation than the conventional mooring. They distribute wastes over a considerably larger area than those secured by a multiple point system. The material used may be either concrete blocks or sand filled bags.



Single point mooring using concrete blocks (Source: CMFRI Hand book on Open Sea cage culture)

Single point mooring using sand filled bags (Gabbion box)

Multipoint mooring is most commonly used mooring system. These systems retain the cages in one particular orientation. These systems use more chains/ropes to adopt the position of cages with least resistance to prevailing wind, wave and current forces. Orientation of cages depends on the nature of the site and group configuration of the cage



Multi point mooring (Cluster mooring at Karwar)

Stocking

Stocking appropriate size and number of fish seed in cages is very crucial for the success of cage farming. After allowing the hatchery produced spawn to grow for a period ranging from 30 to 60 days, fish seed can be stocked in cages. Nursery rearing of seed is essential for all species and it can be done as a separate activity, in land based nursery ponds or hapas held in ponds or in floating nursery cages,

by individuals or groups at different localities to support sea cage farming with ready to stock fingerlings. Healthy, uniform-sized fingerlings should be procured for stocking in cages. The fingerling stocking details are given below

Species	Stocking Size (Length/ Weight)	Stocking Density (Nos./ m^3)
Cobia	15 cm/ 35 g	8-10
Pompano	10 cm/ 35 g	30-40
Seabass	10 cm/ 30 g	30-40
Grouper	15 cm/ 40 g	15-20

Feeding

Feed accounts for 60% of input cost in cage farming so is the importance to manage the feeding. Marine fishes need a diet rich in protein (35-40%) for optimal growth. The size of the feed pellet should be adjusted according to the growth of the fish. The usual feeding amount is 10% of body weight for juvenile fish, but can be reduced to 3% of body weight as culture progress**.** A feed with an FCR of 1:2 is advisable. Feed should be given as per the recommended ration since overfeeding leads to wastage, economic loss and environment pollution.

Feeding rates, frequency of feeding and time of feeding are important factors to be considered in cage farming. Feeding rates and frequencies are related to age and size of the fish. Fish larvae and fry need to be fed on a high protein diet more frequently.

When fishes grow bigger, feeding rates and frequencies can be reduced. Feeding fish is a labour-intensive activity and the frequency must be adjusted in such a way that it is economically viable. Generally, growth and feed conversion increase with increase in feeding frequency. Feed consumption is also influenced by time of day, season, water temperature, dissolved oxygen levels and other water quality parameters. Moist feeds or pellet feeds can be used based on requirements and availability.

Harvesting the cage farmed fishes

The ease of harvesting cage farmed fish is one of the reasons to choose cage culture. Harvest of fish in cages is less labour intensive and can be done partially or completely based on market demand. Harvesting can begin when a significant portion of the fish reach a size the marketable size. At the time of harvest it is advised to record the total no of fish harvested and their total weight. The cage farmed fishes are primarily sold through local fish markets or at farm gates and fetch a premium price owing to their superior quality and freshness.

Harvested Asian seabass

Harvest of pompano and snapper

Good management practices in cage farm management

1. Routinely monitor cages for escapement and properly maintain cage nets so as to prevent fish escapes
2. Optimize feeding protocols and to use good quality feeds
3. Use appropriate stocking densities and employ techniques to minimize physiological stress to cultured organisms
4. Growth rates of cultured fishes should be monitored at appropriate time intervals
5. Cage nets should be monitored regularly and necessary repairs should be done immediately

6. Regular net cleaning and net exchange practices should be carried out depending on the site as well as the season. It usually varies from weekly to monthly duration
7. Periodic monitoring of water quality parameters like water temperature, dissolved oxygen, pH should be carried out
8. Mooring system should be monitored regularly and any defects should be rectified immediately
9. Health status of fishes should be assessed while feeding
10. Implement cage rotation or fallowing
11. Site facilities in areas with sufficient flow rates and avoid areas that may impact sensitive ecosystems (e.g., coral reefs)
12. Always monitor the effects of cage site on the nearby environment
13. Practice IMTA (integrated multitrophic aquaculture)

Further reading

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Fish nutrition and Feed Management in Marine Cage Farming

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Central Marine Fisheries Research Institute (CMFRI) has initiated cage culture in India for the first time and marine cage was successfully launched at Visakhapatnam, in the east coast of India in 2007. CMFRI has taken a lead in popularizing finfish cage culture in India. In the last decade, CMFRI has made significant contributions to mariculture and hatchery technology of high value fin fish species. Marine cage culture is being popularised by ICAR-CMFRI throughout the maritime states of India by providing technical guidelines and by conducting training programmes for fish farmers, fishermen and entrepreneurs. Presently commercial marine cage farming of Cobia, Seabass, Silver pompano, Red snapper, Grouper and lobsters are being propagated by CMFRI

Nutritionally balanced Feed and good quality seed are the major prerequisites for any aquaculture practice. The economic profit of the culture practise is mainly determined by the quality and quantity of the fish feed used. In addition, nutritional status of the feed is one of the crucial factors influencing the growth and immune function of any organism.

Basics of fish nutrition:

Basically there are two types of feeds being used in marine fish farming such as formulated feed and low value fishes. Among them the formulated feeds are more appropriate for successful farming of marine finfishes due to the nutritional quality, storage convenience, lesser prise and continuous availability.

Quantity of the feed is determined by the Nutrients present in the feed. The major macronutrients in fish feed includes proteins, carbohydrates and fats. Protein is considered as the building blocks of any living organism and in addition it helps in the growth of the organism. Amino acids are the basic units of proteins. There are mainly 10 essential amino acids which cannot be synthesised by the fish and 10 non essential amino acids that need not be supplemented through the feed.

Carbohydrates are feed ingredients that are mainly meant for supplying energy for the routine physiological activities of the fish. They are having less importance in marine fish feed formulation, but mey help in gelatinisation and pellet formation in extruded pellets. Fat is another major energy yielding component in fish feed. Fatty acids are the basic units of fat. Essential fatty acids are mainly required for fish growth. Fats yield 2.5 times more energy than carbohydrates and are thus the major source for the energy inputs for fish feed.

Vitamins and minerals are the important micronutrients required for the feed formation. Fat soluble vitamins and water soluble vitamins are the major vital inputs for any organisms. Macrominerals like Calcium and Phosphorus are required in higher quantity and micronutrients such as Copper, Cobalt, Iron, Sulphur, Iodine, Magnesium, Manganese and Zinc are required in micro level in fish diet.

Formulated feed preparation:

There are mainly two types of ingredients used for fish feed preparation such as Protein rich ingredients and carbohydrate rich ingredients. Protein rich ingredients include fish meal, meat products and oil cakes. Fish and fish bye-products acts as animal origin input and oil cake as plant origin input. Generally for marine finfishes fish bye-products such as fish meal and dried fish powder are used as the major protein sourse. Cereals and cereal bye products are the important carbohydrate source for fish feed formulation. Fish feed formulation is the process of mixing different feed ingredients having varying protein, carbohydrate and fat proportions at appropriate ratios to form a nutrient balanced feed. In addition minerals, vitamins and other feed additives are added as feed supplements. Feed production technology is developed mainly for producing sinking pellets, slow sinking pellets and floating pellets. Sinking pellets are mainly used for omnivorous and bottom duelling fishes. Slow sinking pellets and floating pellets are used for column feeders and surface feeders. Extrusion technique is mainly used for producing floating pellets. Generally formulated feeds give better stability, palatability, acceptability and food conversion ratio (FCR) than the low value fishes. More over they are more economical and environmental friendly.

Low value fishes:

Low value fish feed resource includes cheaper fishes such as oil sardine, lesser sardine, rainbow sardine etc. These fishes are fed to the reared fishes either by cutting manually or by chopping with chopping machine. But they yield lower FCR generally up to 6:1 to 17:1 but in a well managed farm with minimum feed wastage, the FCR can be reduced up to 2:1. The major concerns associated with feeding low value fishes are environmental pollution, enhanced cost of production. Continuous exploitation of these natural resources may lead to over exploitation and depletion of the common recourses.

Fig 1. Pellet feed and low value fish used for feeding fish stocked in marine cages.

Feed Management:

The major factors associated with any feed management practice are quality of feeding and the quantity of feed provided to the system. Proportion of the nutrients, stability of the feed and palatability of the feed are the major criteria that can determine the quality of the feed. Feeding rate, Feeding frequency and the time of feeding are important for determining the quantity of feed to be provided for the fishes. Generally when the fish size increases feeding rate need to be reduced. In

usual practices the juveniles are provided with the feed @ 10% of body weight initially. Which can be reduced to 2 to 3% as the fish grows. The feeding rate is also affected by the water quality parameters such as temperature, salinity, ammonia etc. Time of feeding is also an important factor that determines feed utilisation in cage culture practices.

The total ration for each day need to be split into 2 to 4 portions for better utilisation of feed. Generally for fishes, feeding is restricted in the day time and for nocturnal animals like crustaceans (prawns, crabs and lobsters) majority(70%) of the feed need to be given in the late evening hours (18hrs to 19 hrs). Periodic sampling and growth measurements are at most important for feeding and feed calculations. Generally weekly or biweekly samples can be done by collecting 30 to 50 number of fishes for noting the increment in weight and length of the fish. The total biomass of the cage farm can be calculated by considering the average weight of the fish, total number of fish stocked and the approximate survival. From the total biomass obtained, the daily ration of the feed can be depicted.

Fig 2. Feeding fishes stocked in a marine cage

Storage of feed:

Feed storage is another important criteria considered for the success of the fish farming. Low value fishes can be collected and kept in cold storage or deep freezers for months together, but for small scale farming fishes preserved in ice can be used for only for few days. Generally cheaper priced fishes can be collected during fishing seasons when fish landings are more and fish price is minimum and can be preserved for lean seasons.

Formulated feed can be stored in places where moisture content is less. The feed bags can be stalked on racks above from the ground. Feed need to be protected away from Fungus and mold formation, pests and rodents.

Feeding practices of Cultivable fishes in cages:

Presently only few fishes are cultivated in marine cage culture systems which includes Cobia, Pompano, Seabass, Grouper, Red snapper and Lobster.

Cobia:

Cobia is one of the most preferred marine fish for cage culture practices because of its faster growth and good meat quality. The culture practices of cobia include nursery rearing and cage culture phases. The culture is mainly based on either low value fishes or commercial feed. This fish requires a high protein diet for their growth and physiological activity because of their fast moving nature. The Optimum dietary protein and lipid level in juvenile cobia is 45 % and 5 % of dry weight. Juveniles can be fed @ 10% body weight. For cage culture purpose freshly chopped oil sardine @ 6% of biomass per day can be given for the first 3 months. The feeding rate can be reduced by 2% in every 3 months for the remaining culture period. Feed can be given twice a day (6 hrs and 18 hrs).The reported FCR in cage culture is reported to be approximately 1.4 to 1.8. This fish can grow up to 3 to 4 kg in a year.

Asian Seabass:

Asian seabass or Bhekti is another popular fish recommended for marine fish culture. This fish is highly carnivorous and cannibalistic in nature with prominent differential growth. Cultivation of this fish also requires a nursery phase and a cage culture phase. Cannibalism is observed mainly at a younger stage (1 to 20 cm length) or in the first two months of culture. For nursery rearing chopped and ground trash fish (4 to 6mm^3 size) @ 100% biomass twice daily in the first week at 9hrs and 17hrs which can be gradually reduce to 60% of the second week and 40% of the third week. For weaning the fish to pellet feed, make sound to attract the fry to form an school. Feeding time and place should be fixed for training the fishes to accept the feed. Seabass prefer slow sinking feed and thus the feeding should be done at a slower pace. When the fish are fed to satiation they disappear and thus feeding should be stopped. First few days train them for feeding 5 to 6 times per day then reduce it to 2 times. The nursery rearing period last for 30 to 45 days. Size grading of the fishes is required to select uniform sized fishes for stocking into the cages. CIBA (Central Institute for Brackish water Aquaculture, Chennai) has developed Micro-diet with 45% protein and 12% liquid for Nursery rearing of Seabass.

During the Grow out phase the fish need to be fed with chopped fish twice daily in the morning 8 hrs and afternoon 17hrs @10% of total bio mass in the first 2 months. Then feeding can be reduced to once daily @ 5% of body mass in the afternoon. Feed should be given only when the fish swims near the surface to eat. CIBA (Central Institute for Brackish water Aquaculture, Chennai) has developed a Grow out feed containing 38% protein, 8% fat with an FCR of 1.8:1.

Pompano:

Pompano is a fast and continuous moving fish and thus it requires highly nutritive feed. During the nursery rearing phase fish can be weaned to any type of feed such as Floating pellets, sinking pellets and chopped pellets. Feeding can be done 3 to 4 times a day. CMFRI has developed a floating pellet with pellet size varying from 0.8 mm to 4.5 mm size. For 1g size fish, pelleted feed with Crude fat 6% and crude protein 50% can be provided and for fishes with 250 to 500 g size pelleted feed with crude fat 10 % and crude protein 30 % can be provided. The pelleted feed is reported to provide an FCR of 1.8:1.

For other fishes such as Red Snapper and Grouper, the cage Culture practices are being standardised with feeding trash fish.

Lobster:

Fattening of lobster is practiced on experimental basis by providing whole fish or chopped finfish or shellfish as fish feed. Lizard fish is preferred @10% of the body weight. Feed should be provided early morning (30%) and late in the evening (70 %).

Mussel farming

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Introduction:

Mariculture of bivalves assumes greater importance in meeting the increasing protein demands of the human population. Bivalve groups such as oysters, mussels, and clams are the most important cultivable organisms all over the world. Mussel farming has a long history that dates back to the thirteenth century in France. Worldwide, it is one of the most widely cultivated speciesandis farmed in many areas of the world with the most common species cultured being the European blue mussel, *Mytilus edulis*. The main producers of mussels are countries such as Spain, France Netherlands, Denmark,China, Korea, and New Zealand. The Indian mussel industry is modest and the maximum production attained is about 20,000 t. Of these, *P.viridis* and *P.indica* form the most dominant cultivable species. The Central Marine Fisheries Research Institute (CMFRI) has developed eco-friendly techniques for mussel culture. CMFRI has taken up efforts to popularize mussel culture in all the coastal states of India.

Distribution of Mussels in India

Mussel popularly known as "Kallumekai / Kadukka/ Chippi/Neelakallu" are available along the Indian coast. The green mussel, *P.viridis* is extensively distributed as subtidal and intertidal beds along both coasts. Along the Kerala Coast, the major locations are Koduvally,Mahe,Chombala, Moodadi,Thikkodi, Elathur, Chaliyam,South Beach, Anchangadi, Ethai, Narakkal, Chellanam, Andakaranazhi, Azhikkal, Parimanam, Port Kollam and Neendakara.

Along the Karnataka coasts, the mussel beds are mostly seen in subtidal beds and major resources are located in Uchila, Someswara, Suratkal, Matukopal, Malpe, Coondapur, Byndur, Bhatkal, Dhareshwar, Gokarn, Kodar, Karungadi, Karwar, Angola, and Gangoli.

Biology

Perna viridis is a suspension feeder. This species is an efficient ciliary-mucoid filter feeder, feeding on small zooplankton, phytoplankton, and other suspended fine organic material.

Green mussel shows a rapid growth rate by length of 8mm-13.5mm per month. Under average culture conditions, green mussel and brown mussel attain a length of 80-88mm with 36.4 - 40g weight and 65 mm with 25-40 g in 5 months respectively. The farmed mussels give a better meat yield compared to mussels from the natural bed. Growth by length and weight are probably the most important criteria for assessing the success of the culture system.

The growth of mussels is influenced by a number of environmental factors such as water quality, food availability, settling density, water current, and tidal exposure. Sexes in this species are separate and fertilization is external.The gonad of a mature female can easily be distinguished by its bright orange-red colour from that of the male, which is creamy yellow.

Fertilization is external. After fertilization, it attains pediveliger within 15-35 days. Pediveliger attaches to the settlers with the help of byssus threads and metamorphoses to spat. Spat settlement takes place from July to September and attains seeding size in September.

Farming sites

Site selection

Open sea and estuarine areas free from strong wave action are suitable for farming. Clear seawater with rich plankton production (17-40µg chlorophyll /l,) is ideal for mussel culture. Moderate water current (0.17-0.25m/s at flood tide and 0.25-0.35m/s at ebb tide) will bring the required planktonic food and will carry away the excessive build-up of pseudo-faeces and silt in the culture area. The water should have a salinity of 27-35 ppt. ana d temperature of 26° C- 32° C. Site should be free from domestic, industrial, and sewage pollution.

Open sea farming

In open sea farming, the depth at the site should be above 5m without strong wave action, less turbulent, and with high primary productivity. Long line and raft culture techniques are ideal for open sea farming. Mussels grown on long lines become smothered by naturally settling juvenile mussels and other fouling organisms. Effective utilization of easily available material for the fabrication of longlines and rafts can be done. The disadvantages of this farming are poaching and unpredicted climate changes. Protected bays are ideal for mussel farming as in Karwar.

Estuarine farming

Compared to open sea, estuarine ecosystems with less turbulent and shallow depths (<4m) are suitable for mussel farming. The culture of mussels on horizontal ropes results in high productivity due to the effective utilization of primary productivity. Rack culture is ideal for estuarine conditions. Fluctuation in salinity during monsoon season and pollution through domestic and industrial waste are the main constraints in estuarine mussel farming.

Farming methods

The farming practice of bivalve molluscs is either on-bottom or off-bottom culture methods. The bottom culture system is also called the broadcast technique. The off-bottom culture system,includes the stake or pole method, rack, raft, and long-line method. The rack, raft, and long-line method are also called the hanging or suspended culture technique. The stake and rack method is mainly used in shallow, intertidal waters while the raft and long-line methods are generally utilized in deeper, open waters. The farming techniques practicedin many parts are defined by the geological and oceanographic conditions prevailing in thoseregions. Some of these are described below:

1. Bouchot or Intertidal Pole Culture

It is believed that in France, mussel culture started in 1235 when an Irish sailor Patrick Walton survived a shipwreck on the Bay of Aiguillon. He found that the wooden poles and nets that he had kept for trapping birds attracted mussel spat

settlement. This became the basis for the Bouchot method which is the oldest and the main method utilised in France on the Atlantic and the English Channel coasts. This method, well suited to the large intertidal mudflats facilitated the development of the blue mussel (*Mytilus edulis*) industry in France.The spats are collected on spat collecting ropes made of coir. These spat bouchots are situated offshore and consist of parallel rows of poles with horizontal coir ropes for collecting seeds. When the seeds are a few months old, they are removed from the ropes, placed in mesh tubes, and transferred to bouchots for growth. In this method, ropes with spat attached are wound around large vertical poles (bouchots) in the intertidal zone. A barrier is placed at the bottom of the pole to prevent predators such as crabs from reaching the mussels. The poles are placed perpendicular to the shoreline This method of culture requires large tidal ranges, in order to supply the densely packed mussels with plankton.

2. Stake culture

In Thailand and the Philippines, mussels are grown on bamboo poles (6-8m long) staked at half meter depth and one meter apart or in a circle and tied at the top to form a wigwam structure in soft, muddy bottoms. Mussels (*Perna viridis*) settle on the submerged bamboo stakes. Bamboo poles are often observed to monitor growth and eliminate predators like starfish and crabs. Bamboo stakes are placed in areas where natural spatfall is expected. Mussels are harvested after a growing period of 6–10 months after stocking or when the animals reach 5–6 cm in length. Each pole yields 8-12 kg of mussel. Harvesting is done by hauling up the bamboo poles and loading them into a raft. This culture system also facilitates siltation which makes bays and estuaries too shallow for mussel farming. In the Philippines, a rope strung in a zigzag fashion or rope web method is used. Each unit consists of two bamboo poles 5 meters apart driven into the substratum.

3. On-Bottom Culture

This method is widely used in the Netherlands, Denmark, and Germany. The culture is based on the principle of transferring seeds from areas of great abundance where growth is poor to culture plots in lower density to obtain better growth and fattening of the mussel. The culture plots must have a firm substratum and fewer drifting sand and silt particles. In the Netherlands, the seeds are dredged from Waddenzee. The seeds are laid in intertidal areas to produce mussels with thick shells and strong adductor muscle. In the subtidal areas, higher meat yield and thinner shells are produced fit for the processing industry. The whole process is highly mechanized from the collection of seeds to harvesting and marketing.Waddenzee and Zeeland are important areas for mussel (*M.edulis*) farming.

4. Longline culture

This method is becoming very successful in open-sea mussel farming. A rope is stretched horizontally near the water surface and maintained 1-2 m from the surface with buoys. Mussels are grown on vertical ropes known as 'droppers' which hang from the horizontal rope for a length of 4m. Mussel seeds are collected from natural beds and transplanted onto the ropes into a continuous sock-like cotton tube, which is approximately 17.5 cm in width. Small mussels stripped from the collection ropes are inserted. This cotton sock is then wound around the dropper. The mussels grow and attach to the ropes using their byssal threads and the cotton sock slowly disintegrates and falls away. The droppers are placed a minimum of 0.5 m apart and have at least 4 m of free space from the bottom. In deeper waters,

the gap between the bottom of the line and the sea floor is greater. Anchor ropes extend from each end of the horizontal rope to anchors buried in the mud of the bottom. As the ropes are kept taut, there is no movement around the anchor to disturb the bottom as occurs when boats are anchored.
The density at which mussels can be cultured on long lines could be about 300 per meter but depends on the food availability, which varies from site to site. Mussels grown on longlines can become smothered by naturally settling juvenile mussels and other fouling organisms. For this reason, most farmers prefer to position their farms away from heavy spat settlement areas to avoid layers of spat attaching to larger mussels.

5. Raft Culture
The basic principle of raft culture is similar to long line culture in that the mussels are suspended on droppers but these are suspended from the raft instead of the long lines. The raft itself is anchored to the seabed removing the need for several anchoring systems. Long line culture, however, creates less of a visual impact, and the droppers can be spaced farther apart to maximize the use of the available phytoplankton. Raft culture is more suited to areas of dense phytoplankton and to smaller operations, as there is less scope for mechanical harvesting. This method of culture is used in the Galician Bays in Spain, and Saldanha Bay in South Africa but has been abandoned by the New Zealand industry in favour of long lines.This method has its origin in Spain in the Galician Bay. Mussel seeds (*Mytilus golloprovincialis*) settle profusely in the inter-tidal zone in the coastal waters of Galicia. These rias are sheltered, and nutrient-rich with 3-4 m of the tidal range providingthe ideal environment for suspended mussel culture. These seeds are collected by scrapping the rocks with spade-like steel blades. Seeds can be collected by suspending ropes vertically from the rafts. The length of the mussel ropes varies from 6-9 meters according to the depth of the culture site. As the production is about 10 Kg of mussel per meter of rope, a raft having 600 to 1000 ropes of 6-9 meters may produce 30000 to 90000 Kg of mussel per year.

6. Rack culture.
This is the simplest rope method used for green mussel cultivation in India and the Philippines. The main purpose of the pole is to support the structure. In between these poles, ropes are suspended either vertically or kept horizontally where the depth is a limitation. The construction is labour intensive but the simplicity in harvesting and accessibility of local materials for farming purposes makes it very adaptable under local conditions.Mussel culture is fast becoming popular in the Malabar area since 1997 following the success achieved by CMFRI in rearing green mussels by rack culture in the backwaters. The simple methods employed for mussel farming were transferred to progressive farmers who took up mussel culture in the backwaters. Soon they found the venture profitable. Demands came from new entrepreneurs for training and mussel farming spread from Kasaragod to Ponnani. Mussel culture in the backwaters of Kerala was first started in Padanna and Cheruvattur Panchayats in Hosdurg Taluk of Kasaragod district. Later it was taken to Elathur in Calicut district and Vallikunnu and Ponnani in Malappuram district.Some of the constraints are regarding the availability of seeds.Mussel farming is more than two decades in India. This is a low investment activity with very good returns. If promoted properly, mussel farming can be used as a tool for

women empowerment in the coastal areas and can stimulate healthy socio-economic development in the area. Better post-harvest technologies can develop attractive value-added products. Since very good export markets are available for mussels there is further scope for extending the farming practice to suitable areas.

Seeding the mussel ropes

The mussel seeds are collected from areas free of pollutants from the subtidal areas. After removing the fouling organisms, the seeds are washed thoroughly in seawater. About 500-750g of seed is required for seeding on a one-meter length of rope. The ideal size of the seed is 15-25mm. The length of the rope is decided by considering the depth where the raft/ rack is positioned. While suspending the seeded rope on the rack it must be tied in such a way that the upper seeded portion of the rope should not get exposed during low tide and the lower portion should not touch the bottom of the estuary.

Nylon/coirropesare used for seeding. Cotton netting of the required width and length is placed on the floor and the required quantity of seed is spread over the net from one end to another. The rope is kept above the net and is tightly stitched in such a way that the seeds spread uniformly around the rope. The cloth will degenerate within 2-3 days. By this time the seeds will secrete byssus thread and will get attached themselves to the rope.To avoid slipping the mussels, knots are made on the seeded rope at a distance of 25cm.

Rearing the mussels

The seed, which gets attached to ropes, shows faster growth in the suspended column of water. If the seed is not uniformly attached, the crowded portion always shows slipping. To avoid slipping, a periodical examination of seeded rope and thinning of the same is essential. The seeded mussel on the upper portion of the rope shows faster growth due to the abundance of phytoplankton. For better growth, the seeded ropes should be spaced at a distance of 25 cm.

Management

Constant vigil is required to see that the raft/rack is in position. Thinning may be done if necessary to avoid the loss of mussels and to provide enough growing space. Periodic removal of fouling organisms like barnacles, polychaetes, and ascidians is to be done for improved growth.

Harvest, product development, and marketing

Harvest is done when the mussels reach the marketable size and this s time before the onset of the monsoon in the estuarine areas. Normally harvest season is from April to June. The mussel ropes are collected manually and brought to the shore for harvest andwashed thoroughly to remove grit and slit. The mussels separated from the ropes are maintained in re-circulating seawater for 24hrs and washed again in fresh seawater. This method of depuration is effective in reducing the bacterial load of the mussel meat by 90%. The depurated mussels are then mainly

sold through the local market as live shell-on mussels. At present processing units use only a small quantity of cultured mussel. New strategies need to be developed to fully exploit the domestic market.To avoid the risk of consuming mussel meat and to increase the quality of mussel, depuration is essential. During the process of feeding, mussels accumulate all suspended biological materials including harmful microorganisms. Before the product reaches the market, these materials have to be removed from their gut. The process of such purification is called depuration. Here the mussels are placed for 24 hours in cleaning tanks under a flow of filtered seawater. About 10-20% of the seawater is continuously replaced. At the end of 12 hours, the water in the tank is drained and mussels are cleaned with water to remove the accumulated faeces. The tanks are again filled with filtered seawater and the flow is maintained for another 12 hours. Then the tanks are drained and flushed with a jet of filtered seawater. The mussels are held for about one hour in 3 ppm chlorinated seawater and then washed once again in filtered seawater before marketing.A variety of products has been developed in India from mussel meat. These products have been developed by R & D activities of CIFT, Kochi. In the retail market, few mussel products are available. The latest product in the line is the condiment that incorporated ready-to-eat fried mussel meat in vacuum packs.

Integrated multi-trophic aquaculture IMTA

Conventional aquaculture is often associated with ecological problems when done on large scale. Many of these issues can be solved with the environmentally friendly approach of Integrated multi-trophic aquaculture (IMTA). This system provides the by-products from one aquatic species which serves as input for the growth of another. Thus along with finfish farming in cage culture,one can grow seaweed for inorganic extraction and bivalve for organic extractives and thus balance the environment(bio-mitigation). This is the basis for economic stability and sustainability of the farming system.

Algae, being at the bottom of the food chain in the ocean can be produced with little effort and very sustainably.The multi-trophic sub-systems integrated into IMTA refer to the more intensive cultivation of the different species in proximity of each other, linked by nutrient and energy transfer. While selecting the species for farming one has to see if the organism for farming is native and adapted that has a matching role with other species in the system. The species farmed should be adapted to the environment and also has a market value

Diseases of Mussels

In large-scale farming of mussels, it was noticed that diseases were mainly due to the use of stressed seeds, poor environmental conditions, clustered farms with little flushing, high salinity, and temperature with *Perkinsus olseni* infection. In the farm management practice, the carrying capacity of the waterbody should be determined and also reduce the area of each farm unit and avoid the concentration of farms in an area. Artificial structures reducing the flow of water should be avoided. Only

good quality seeds should be used and the monitoring of the farms should be done regularly.

Training in seeding of mussel ropes

Training in rack construction

Mussel racks at Padanna

Harvest of mussels at Iringal, Badagara

Harvest of mussel from cage farm (IMTA)

Mussels infected with *Perkinsus*.

Seaweed Farming

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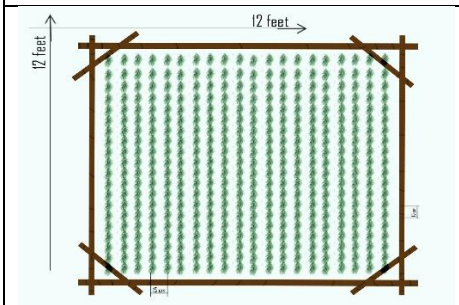
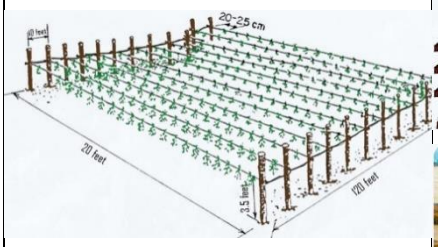
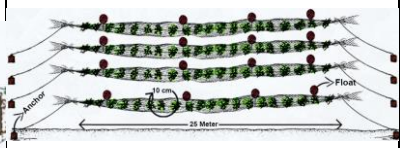
Seaweed farming is a climate resilient aquaculture practice. This cultivation does not need land, freshwater and fertilizers. It is a sustainable diversified livelihood option for coastal communities. It reduces the effects of oceanic eutrophication and acidification and oxygenates the seawater for a healthy ecosystem. Seaweeds are valued commercially for their cell wall polysaccharides such as agar, algin, carrageenan etc. and for their bioactive metabolites, manure and fodder. They have a variety of commercial applications in food, pharmaceutical, cosmetics and mining industries. Some seaweeds are also gaining importance as healthy food for human consumption. World seaweed production was 35.1 million tonnes wet weight with first sale value estimated at 16.5 billion USD (FAO, 2022). In India, nearly 20,000 tonnes wet weight of seaweeds per year are being harvested from the natural seaweed beds (species of *Sargassum, Turbinaria, Gracilaria* and *Gelidiella* by nearly 5,000 families in Tamil Nadu) (FRAD, CMFRI, 2020).

Farming Techniques

	Monoline method	**Tube net method**
  In places which are calm and shallow, floating bamboo raft method (12 × 12 feet bamboo poles) is ideal.	  In places characterized by moderate wave action, shallow depth and the presence of less herbivorous fishes, monoline method of seaweed farming is ideal.	  The tube net method is being adopted in places with higher wave actions.

In India, seaweed farming is being carried out with *Kappaphycus alvarezii*. It is one of the economically important red algae, which yields carrageenan, a commercially important polysaccharide. Farming of *Kappaphycus alvarezii* by the fisherfolk of Tamil Nadu coast has touched highest yield of 1,500 tonnes dry weight in 2012-13. However, the production sharply declined after 2013 due to mass mortality. Currently, around 400-500 tonnes dry weight per year is being produced. Around 1,000 families are involved in seaweed farming in Tamil Nadu coast.

Economics of *Kappaphycus* Farming

Bamboo raft and monoline method of seaweed farming are being predominantly adopted in Tamil Nadu coast. Tube-net method is ideal in coastal states like Andhra Pradesh and Gujarat. The crop duration for *Kappaphycus* farming is 45-60 days. In a year, four to six crops or cycles (6 to 9 months) can be harvested depending upon the climatic condition. Seeding of 150g grows up to 500 to 1000g in 45 days. The average seed requirement for one raft of 12 x 12 ft size is 60 kg, whereas for a tube-net of 25m length it is 15 kg. The average dry weight percentage of the harvested seaweed is 10 per cent. At present farmers receive Rs. 12/- and Rs. 70/- per kg for fresh and dried seaweed respectively. The economics of *Kappaphycus alvarezii* farming as analysed by ICAR-CMFRI is as follows:

- Seaweed production: 1,000 kg/raft/year minus 240 kg which is used as seed material for 4 crops/year = 760 kg (wet weight)
- Price of seaweed: Rs. 70/ kg/dry weight (Dry weight = 10% i.e., 76 kg)
- Total revenue generated: Rs. 5,320/year/ raft @ Rs. 70/kg/dry weight
- Total cost of production (including capital cost): Rs. 2,000/raft/year
- Net revenue: Rs. 3,320/raft/year (Rs. 5,320 minus Rs. 2000)
- A person can handle an average of 45 rafts (12 ft x 12 ft)
- **Total Net revenue (30 rafts) in dry weight = 30 x Rs 3,320 = Rs. 99,600/year**
- **Net revenue from one hectare (400 rafts) in dry weight = Rs. 13.28 lakhs/year**.

Integrated Multi-Trophic Aquaculture (IMTA)

Intense fishing pressure along the coastal waters, coupled with negative impacts of climate change has lately started impacting the livelihoods of fishers. While harvests are dwindling, the demand for marine fish is increasing steadily owing to its crucial role in ensuring food and nutritional security of the population. This necessitates augmenting marine fish production through farming of promising commercial species of fish in the sea. Realizing this important priority, the ICAR-CMFRI has developed and standardized the technologies for seed production and farming of marine finfishes and shellfishes in open sea cages. One of the anticipated issues while expanding the sea cage farming is the increased organic and inorganic load in the water and consequent disease problems. In this context, the idea of bio-mitigation along with increased biomass production can be achieved by integrating different groups of commercially important aquatic species which are having varied feeding habits. This concept is known as Integrated Multi-Trophic Aquaculture (IMTA) which is gaining global importance in recent times. The ICAR-CMFRI has

successfully conducted trials and demonstrated the IMTA by integrating seaweed with sea cage farming of marine finfishes/shellfishes in Tamil Nadu, Gujarat and Andhra Pradesh. This has resulted in increased production of seaweeds which has improved the livelihood of farmers and has also contributed to the carbon credit of the country.

The ICAR-CMFRI is promoting cage farming of cobia, a high value marine fish since 2010. To achieve environmental sustainability and economic stability, an innovative idea of integration of seaweed with sea cage farming of cobia was demonstrated during 2014-17 at Munaikadu, Palk Bay, Tamil Nadu. A total of 16 bamboo rafts (12× 12 feet) with 60 kg of seaweed per raft were integrated for a span of 4 cycles (45 days/cycle) along with one of the cobia farming cages. The rafts were placed 15 feet away from the cage in a semi-circular manner, so as to enable the seaweed to absorb the dissolved inorganic and organic nutrient wastes which moves along the water current from the cage.

Currently through IMTA, seaweed rafts integrated with cobia farming cage had a better average yield of 390 kg per raft, while in the non-integrated raft the yield was 250 kg per raft. **An additional yield of 140 kg of seaweed per raft (56% additional yield) was achieved through the integration with the cage farming of cobia. An additional net income of Rs. 62,720/- (896 kg × Rs.70/kg) was realized through integration of seaweed rafts with cobia cage.**

The carbon sequestered into the cultivated seaweed in the integrated and non-integrated rafts was estimated to be 497 kg and 319 kg, respectively. Hence an **additional 178 kg carbon credit was achieved through the integration of 16 seaweed rafts (4 cycles) with one cobia farming cage (per crop). In one hectare of area, a total of 20 cages of 6 m diameter can be integrated with 320 bamboo rafts (12× 12 feet) @ 16 bamboo rafts per cage.** IMTA is an eco-friendly option ensuring sustainable income to the coastal fishers. It is also one of the significant mitigating measures for reducing the adverse impact of climate change and also earns carbon credit to our country.

Suitable sites for seaweed farming

In view of the emerging importance of seaweed mariculture, an all-India preliminary survey for selection of sites suitable for seaweed farming was conducted by ICAR-CMFRI using around 15 parameters along all maritime states of India. **From this survey a total of 23,970 ha area in 317 locations along 8 maritime states, Diu and Lakshadweep were identified as having potential for seaweed farming along the Indian coast** (Johnson, *et al.,* 2020). The ICAR-CMFRI has also brought out the "Decision support spatial suitability map for Seaweed Farming in India" (Divu, *et al.,* 2021). These studies will pave the way for proper marine spatial plans for undertaking seaweed farming.

Way forward

- The Indian requirement of agar and alginate is about 400 tonnes per annum and 1,000 tonnes per annum respectively, whereas only 30% and less than 40% respectively of it has been produced indigenously. The Indian requirement of carrageenan is 1,500-2,000 tonnes per annum. Taking the demand on agar, alginate and carrageenan, the total annual seaweed requirement on dry weight basis is 4,000 tonnes of agar yielding algae; 5,000 tonnes of alginate yielding algae and 4,500- 6,000 tonnes of carrageenan yielding algae. Hence to attain self-sufficiency in seaweed-based products, large scale farming needs to be promoted.
- Scarcity of quality seed material for *Kappaphycus* cultivation in coastal areas and quality seed materials of native species such as *Gracilaria edulis, Gracilaria dura and Gracilaria debilis* especially after monsoon rains is the major bottleneck for seaweed development in our country. To address this issue, import of high-yielding species/ varieties and establishment of seed banks for improving the availability of quality seed material to support farming activities is the need of the hour.
- Developing *in vitro* cell culture techniques for selected seaweeds is crucial as it will facilitate year-round mass supply of seed materials maintained under controlled conditions.
- Strain development and hybridization of *Kappaphycus* and *Gracilaria* through protoplast fusion techniques are envisaged for production of fast growing, productive and high-temperature-tolerant seaweed seed materials.
- Formation of seaweed-based Fish Farmers Producers Organizations (FFPOs) is the need of the hour to economically empower the seaweed farmers and enhance their bargaining power.
- Appropriate financing and insurance cover against crop losses due to natural calamities is essential to further promote seaweed farming in Indian waters.
- Currently the Indian seaweed cultivation is located in near-shore waters. To experiment the offshore farming techniques, policy interventions and its techno-economic viability is essential. Integration of seaweed along with cage farming can be promoted wherever possible for bio-mitigation.
- Seaweeds as healthy food for human consumption has to be promoted through awareness campaigns and seaweed food festivals.

The large-scale mariculture of seaweeds is a green technology which will improve the livelihood of coastal fishers and at the same time mitigate major greenhouse gas and can check ocean acidification.

Integrated Multitrophic Aquaculture

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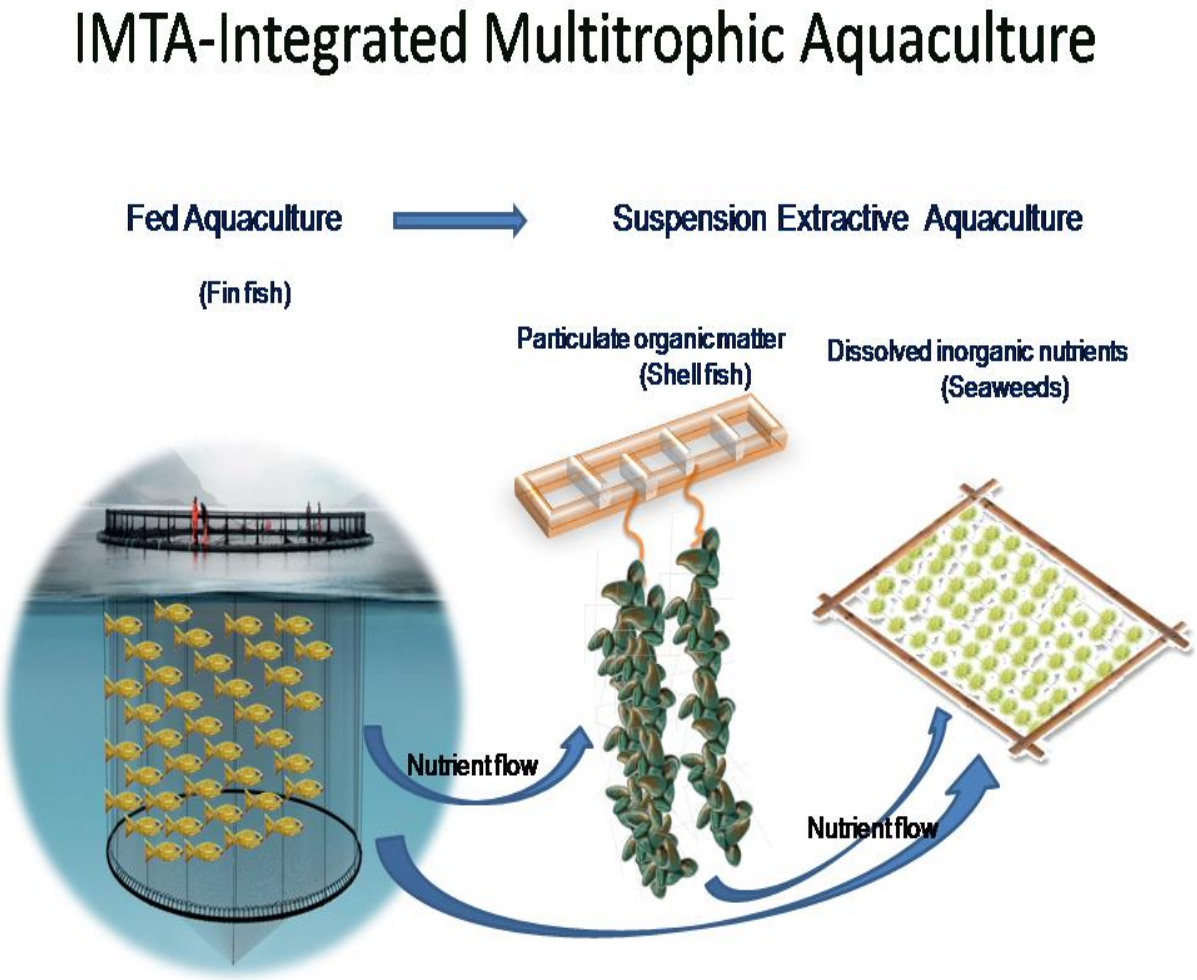
Despite this effort, intensive aquaculture production still releases high amounts of nutrients and organic wastes into the environment that can cause eutrophication of coastal areas and other aquatic systems (Sarà et al., 2018a). This is because only about 20–40% of the nitrogen (Oliva-Teles et al., 2020) and less than 50% of the energy intake (Peres and Oliva-Teles, 2005) are retained by the species produced. The recognition of the significant environmental and social impact of intensive production increased the interest in alternative sustainable practices, such as integrated multi-trophic aquaculture (IMTA) (Alexander et al., 2016a; Sarà et al., 2018b).

"Integrated" refers to intensive and synergistic cultivation, using water-borne nutrient and energy transfer. Integrated aquaculture provides nutrient bioremediation capacity, mutual benefits to the cocultured organisms, economic diversification and increased profitability. It is necessary that a successful sustainable integrated farming system mimics in a way similar to functions of the natural ecosystem. "Multi-trophic" means that the various species occupy different trophic levels, i.e., different (but adjacent) links in the food chain. Integrated multi-trophic aquaculture aims at the integrated production of aquaculture species of different trophic levels under a circular economy approach, minimizing energy losses and environmental deterioration (Food and Agriculture Organization [FAO], 2009; Chopin et al., 2012; Hughes and Black, 2016; Buck et al., 2018). The species integrated belongs to different trophic levels sharing complimentary chemical and biological processes hence have balancing effect on ecosystem and improves the overall health. In integrated multi-trophic aquaculture (IMTA) the by products, including waste, from one aquatic species as inputs for another species integrated in the same system. These systems thereby provide environment remediation (biomitigation), economic stability (improved output, lower cost, product diversification and risk reduction) and social acceptability (better management practices).

There are multiple configurations of IMTA systems, integrating the production of vertebrate and invertebrate species and macroalgae. Cultivated organisms are fed aquatic species, like fish or shrimp, and species extracting the organic and inorganic matter from the water. Species extracting the organic matter may be mussels, oysters, clams, sea urchins or polychetes. Species extracting the inorganic matter, such as macroalgae (e.g., *Kappaphycus*, *Gracilaria*, *Ulva*, *Saccharina*, *Laminaria*), capture and use the inorganic nutrient

wastes. Fed organisms (mainly carnivorous fish and shrimp) are nourished by feed (commercial diets or trash fish). Extractive organisms (seaweeds and bivalves), as the name implies, extract their nourishment from the environment. Bivalve (e.g., mussels, oysters and clams) build their own body while degrading suspended organic particles (uneaten feed, phytoplankton and bacteria) that they filter from the water. Seaweeds use sunlight to build their biomass, while assimilating dissolved inorganic nutrients removed from the water. These extractive organisms can turn pollutant nutrients into commercial crops and loaded effluents into clean water.



Examples of IMTA

Israel	Seabream+Ulva Abalone+Fish+Ulva Abalone+Fish+Mollusc+Ulva
Chile	Gracilaria+Turbot Macrocystis+Salmon

China	Shrimp+Crab+Seaweeds Mussel+Scallop+Laminaria/Undaria Fish+Gracilaria
Hawaii	Shrimp+Gracilaria
USA	Salmon+Porphyra
Norway	Salmon+Mussel+Laminaria
Philippine	Seabream+Eucheuma/Gracilaria
Australia	Shrimp+Oyster+Gracilaria
India (CMFRI experiments)	Cobia+Seabass+ Seaweeds/Green mussel- CMFRI, Karwar Cobia+Kappaphycus- CMFRI, Mandapam CMFRI, Veraval

IMTA allows the creation of more sustainable production systems because wastes of fish/shrimp production are valued as a resource rather than considered a burden or pollution. This contributes to environmental sustainability and a more efficient use of resources, while favoring economic diversification (product diversification, bringing company stability through risk reduction), and social acceptability (best management practices).

The concept of integrated aquaculture constitutes an essential element in Coastal Zone Management, aimed at reducing, in an economically and socially beneficial manner, the adverse environmental impacts of aquaculture (freshwater, saline or marine) on the coastal environment. Recycling of waste nutrients by algae and filter-feeding shellfish is the most likely way to economically improve world mariculture sustainability. Integration with seaweeds and/or filter feeders is often the only economically feasible alternative for waste treatment in open-water aquaculture systems.

IMTA can promote aquaculture sustainability, with environmental, economic, and social advantages. This can be achieved through nutrient cycling, increased economic resilience arising from increased production efficiency, product diversification, and potential price

Integrated multi-trophic demonstrations at Karnataka

Under All India Network Project on Mariculture, a fishermen self-help group, Shri Vigneswara Prasanna, under the technical guidance of Karwar Regional Station of ICAR-CMFRI carried out the IMTA in two 6 m diameter galvanised iron (GI) marine cages at Harwada, Uttara Kannada. These cages were stocked with seabass (*Lates calcarifer*) and silver pompano (*Trachinotus blochii*). Seeded ropes (16 ropes each) of green mussel, *Perna viridis* were also tied on the outer frame of these two marine cages. A total of 300 kg of seabass, 100 kg of pompano and 150 kg of green mussels were also harvested after 135 days of culture.

IMTA demonstration was also carried out at Koderi, UdupI. Under the technical guidance of Karwar Regional Station a fishermen self-help group, Karavali friends, carried out integrated farming of seabass and green mussel (8 ropes) in a 6 m diameter GI cage. A total of 398 kg of seabass and 60 kg of green mussels were harvested from Integrated Multitrophic Aquaculture (IMTA) from this demonstration cage.

Integrated multi-trophic aquaculture has additionally been recognized as a contributor to reducing public opposition toward intensive aquaculture (Ridler et al., 2007; Alexander et al., 2016b; Buck et al., 2018). Nevertheless, there is still a pressing need to enhance societal awareness, perception, and acceptability of aquaculture products, and disseminate sound and rigorous information to consumers about the aquaculture industry and its environmental sustainability.

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Fundamental flaws and its impact on sea cage farming

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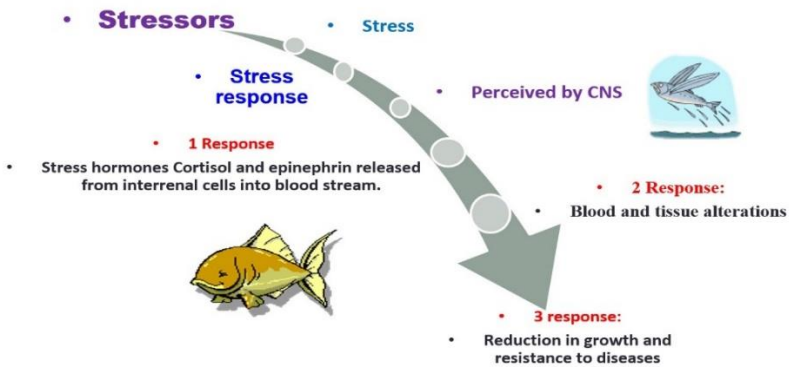
Open sea cage farming is a particularly unique form the other aquaculture system in that the farmer has slight control over the water moving through the culture system. Here in the open sea cage farming farmer has almost no control on the environment, that directly affect water quality or there is no control over the surrounding ocean ecosystem of the cage. It is very important that the farmer acquires a good knowledge about how disease occur and its pattern operates in open sea cage farming and this will help the farmer to control more disease in system.

The disease is a regular part of open sea cage farming. The interaction of marine fish (host), environment, and pathogen produce chronological patterns of disease within a geographical region. Open sea cage farming is to be done in the ocean and this is new ecosystem, often with unique sets of interrelationships among the host, pathogen, and environment. Chronological patterns for the same disease within a sea cage area can be quite different from those in the surrounding environment.
Stress is a critical factor in fish health. It is so important, in fact, that scientists have studied it in detail, both in the wild and in captive fish. (https://www.petmd.com). The primary rule to remember with stress is that, as the saying goes, prevention is better than cure. The things that cause stress by "stressors" in a fish's life include overcrowding, handling, a poor or unfavorable environment, inappropriate or aggressive fish sharing the same tank and, in the wild, predators.

All of these cause fish to react in different ways depending on the type and amount of stress.



Fish have evolved and live in a relatively stable environment. Their stress responses are consequently better at handling short-term trouble and are not as well-suited to long-term environmental stressors. Unfortunately, both types of stress can cause problems. The management of diseases involves an understanding of pathogen, host and environment, and, where possible, management of these interactions for the benefit of the cultivable marine fish for all toward a stable balance.
Although decisions must also consider the cost benefit to production. The future of open sea cage disease management will involve further defining and quantifying the disease agent/host/environment interaction for each disease of concern, within an economic context.
Most discussions on diseases in open sea cage culture focus on diagnosis, pathology, disease causing agent or chemical treatment. However, other aspects of production, disease are still often unclearly approached and defined. Even though most pathologist/disease experts are advising the good management practices i.e., not stressing the cultivable fish and maintain good environment in open sea cage culture that have been avoid the disease outbreak. These are often become more problematic to the culture system or farmer when real problems occur. When a disease outbreak occurs, most of the experts focus on pathogen isolation, identification and disease treatment but they forget the basic interactions of the host/pathogen/environment.
In this context fundamental flaws and its impact on open sea cage culture explained. This will be benefitted to the farmers, entrepreneurs and stakeholders.

Common flaws made by farmers/fishermen include:

- Site selection: picking with pre-existing problems.
- Cage construction with poor material.
- Stocking with poor quality seed.
- High stocking density per cage.
- Feeding with poor quality feed/meat/trash fish.
- Overfeeding.
- Poor handling of fish and lacking knowledge in cage farming.
- Regular monitoring of cages and not observing fish health conditions.
- Lacking knowledge in identifying basic problems like initial disease/ unhealthy fish in cage farming.

Disease impact on open sea cage farming:

Marine fish in cages are often exposed to many wild/new pathogens, depending upon the many factors of disease causative agents, determinants in the culture system cause lesser/greater disease than in the wild. Disease can be defined as anything that physiologically affects a fish and reduces production efficiency (nsgl.gso.uri.edu). Disease is not an "all or nothing event" and can be viewed as a continuum or spectrum in the quantity of lost production.
Pathogens are often existing in the surrounding environment, when there is favourable conditions immediately pathogen will multiply rapidly and increase in abundance and its impact toward the disease outbreak and finally it will lead to mortality. However, even before clinical symptoms are recognized, there may be

performance by the fish like reduced feed intake, dark coloration of the body, reduced growth and surfacing etc. Alternatively, there is increase/proliferate in disease causative organisms this can lead to further worst conditions in culture systems. Disease causative organisms are broadly classified as bacteria, parasites, virus and fungus etc.
Cage culture is one of the most intense forms of aquaculture and can be prone to disease problems. Farming fish in cages can therefore increase the risk of outbreaks of diseases, including those caused by opportunistic parasites. However, disease issues can be controlled through selective breeding and preventive measures; including sanitation, vaccination, and general fish health management.

Disease diagnoses in sea cage farming:

Usually, changes in behavior are caused by changes in their surrounding environment or changes in their body (i.e. cultivable fish body). These changes may be the first signs of stress which signs include:

- Cultivable fish reduce taking feed/feeding stops suddenly.
- Surfacing of many fish for gulping air
- Erratic swimming nature during/under stress.
- Heavy mucus on the surface of the body, and discolorations/dark coloration are seen on the skin of the fish.
- Spots, fin erosion, erratic swimming, or other strange behaviors are usually signs of diseases or parasites.
- Dead fish were found on the bottom of the cage later fish float on water

If cultivable fish is taking less food, it indicates/ it could be due to disease, parasitic loads, low oxygen levels, or one of several water quality problems (e.g., ammonia). It may be due to other reason like a sudden change in weather, water turbidity, and low temperatures. Fish at the surface gulping for air is a sign of critically low oxygen levels.

A few dead fish each day in the cage usually indicate some type of slowly spreading disease or parasite problem. This will cause chronic mortalities in cage farming. Suddenly more dead fish in cages each day is a sign of a very serious disease problem. This is called an acute disease. Freshly dead fish with suspected diseases should be diagnosed immediately. Live samples/ freshly dead samples of fish along with water samples should be submitted to the nearest fish disease diagnostic lab. Disease diagnosis should be done by a disease specialist with a set of protocols in dedicated sampling, in order to develop a baseline for future comparison.

Treatment in cage farming:

Open sea cage farming is a complex form of aquaculture. Fish are crowded and susceptible to many kinds of diseases. If a disease outbreak occurs, medicated feeds or bath treatments with approved chemicals are accepted to treat fish in open sea cage farming. These are costly and sometimes ineffective in the open sea. If diagnosed as an internal bacterial disease, the most practical treatment is usually to feed a medicated feed (e.g., OTC/ Terramycin as per recommendations).

The key to the prevention of stress and disease is Good Management Practices. This means maintaining good water quality, good nutrition, and sanitation.

- Good water quality involves adequate oxygen levels, maintaining appropriate pH and temperature for the species cultured in cages, and preventing the accumulation of wastes.
- Poor water quality is the most common stressor of cultured fish and will precede many disease outbreaks.
- Feeding a high quality diet that meets the nutritional needs of the cultivable species.
- Proper sanitation is also good management. Wastes that accumulates below the cage is an excellent medium for reproduction of fungal, bacterial, and protozoal agents.
- It is important to ensure adequate space below the cage, as well as good water flow through. This will not allow waste from accumulating below the cage.
- Disinfection of containers and equipment will minimize transmission of disease.
- Immersing fish for 10 - 30 minutes in freshwater mixed with formalin (100-200 ppm), should be depending on the tolerance of the fish species and their size.
- Keep on the monitor during the treatment and be prepared to stop treatment immediately if adverse reactions like gasping for air, strange swimming behavior, etc.
- Treatment should be during the coolest part of the day.

A good way to avoid disease outbreaks is good management practices and water management. Water quality should be monitored on a regular basis and if any changes are found immediate action needs to be taken up. Fish live in water and excessive time out of water will lead to disease-causing stress on them. Other ways of managing disease risks are:

- Skill development training for the farmers, it gives good knowledge and they identify problems early.
- Emergency or partial harvesting can be done farmers can still gain benefits from sick fish.
- Stock the fish, which are genetically less susceptible to diseases.
- Stocking larger fish is always better.
- Regular net exchange of cages and disinfection of nets must be done using bleaching powder or formic acid (3%) in order to eliminate the attachment of fouling and parasitic organisms.
- Prompt usage of available and approved treatments.
- Communication among cage farmers provides support and guidance; aiding the dissemination of information.

Sustainable open Cage farming:

Cages are generally located in rivers, reservoirs, and oceans, and are often in crowded conditions also there are many issues with authorized local bodies. Appropriate locations of cages with fewer densities and local authorized bodies may permit the farmers to increase total production capacities. This will help to improve the interrelationship between cage culture and wild fishery. Intensification of cage farming with integrated farming of other species may increase the production in long run. Development/expansion of cage farming may also provide employment for many people. Skill development training for the farmers and unemployed people

gives good knowledge and it will help capture and culture fishery long-term sustainability.

Future prospects and development:

Farmers are facing many disease problems in cage farming. Globally, sea cage farming has encountered serious economic problems due to bacterial, viral, parasitic infestations, and various other infectious diseases. Even though such diseases are being detected and simultaneously treated with several therapeutic and prophylactic methods.

Probiotics, prebiotics, and immunostimulants are also treated as important values for the control of diseases. The immunostimulants show immunostimulatory effects on cultivable fish. Glucan, chitin, lactoferrin and levamisole for fish and shrimp have been reported. Vitamins B and C, growth hormone and prolactin have also been reported as immunostimulators. At present, the development of vaccines, and bacteriophage against major bacterial species are ongoing.

Probiotics: Probiotics are live microorganisms that confer a health benefit to the host by providing both a nutritional benefit and protection against pathogens. These are working on a principle of competitive exclusion against harmful bacteria. Production of inhibitory compounds, competing for essential nutrients by modulating the immune responses is also done by probiotics. Major groups include Bacillus, Enterobacter, Leuconostoc meseneroides, Lactobacillus, Lactococcus, many yeast species, and Streptococcus in these probiotics.

Prebiotics: These are nondigestible, introduced into food, and help bacteria and beneficial microorganisms flourish.

The most common prebiotics used in fish are

- Inulin,
- Fructooligosaccharides (FOS),
- Short-chain fructooligosaccharides (scFOS),
- Oligofructose, mannanoligosaccharides (MOS),
- Trans-galactooligosaccharides (TOS),
- Galactooligosaccharides (GOS),
- Xylooligosaccharides (XOS),
- Arabinoxylooligosaccharides (AXOS),
- Isomaltooligosaccharides (IMO)

Immunostimulants: The immunostimulants show immunostimulatory effects on cultivable fish. An immunostimulant is defined as a substance that enhances the innate or non-specific immune response by interacting directly with cells of the immune system and activating them. Immunostimulants can be grouped under different agents based on the source, such as bacterial preparations, polysaccharides, animal or plant extracts, nutritional factors and cytokines. Beta-glucans, polysaccharides, lentinan, levamisole, schizophyllan, oligosaccharides, Chitin and Chitosan, muramyl dipeptide, Vitamin C, E, Plant extracts, Cytokines-Leukotriene, Interferon and yeast derivatives are using as immunostimulants in the field.

Phage therapy: Phages are the viruses that invade harmful bacterial cells and disrupt the **t**wo major infection cycles which include the lytic (or virulent) and the lysogenic cycle of their metabolism. Lytic phages replicate inside the host cell and progeny viruses are released causing cell lysis. This capacity to destroy host bacteria sets the stage for the use of lytic phages as therapeutic or prophylactic agents. A method using naturally occurring lytic phages, or their products, as bioagents for the treatment of bacterial infectious diseases, is called bacteriophage therapy.

Bacteriocin: Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of other harmful bacteria, bacteriocins attack bacteria by focusing on the plasma membrane. These are having a lower potential for resistance than antibiotics and also hardiness against ultraviolet and heat, unlike other antimicrobial peptides, they are not toxic to mammalian cells. Lactic acid bacteria produce a bacteriocin, nisin A, which has been used in food preservation and acts as a bacteriocin.

Antibiotics: Antibiotics are routinely applied in bath treatments or mixed with feed to prevent (prophylactic use) and treat (therapeutic use) bacterial infections. The most commonly used antibiotics are Oxytetracycline and chloramphenicol. Teixobactin - A new class of antibiotic capable of, killing "without detectable resistance" was discovered in 2015 (Ling., et al. 2015).

Vaccination: At present, the development of vaccines against major bacterial species is ongoing. Vaccines play a major role in preventing antibiotic abuse. Vaccines against infections have also been introduced recently to give immunity and to avoid handling stress. On the other hand, vaccination should not be carried out too early, as the degree of immunity declines with time. Water temperature is an important factor when deciding when to vaccinate.

The broad-spectrum activity of vaccines plays a vital role as a preventive measure in sea cage farming. However, treatments like the use of antibiotics and probiotics seem to be less effective when new mutant strains develop and disease-causing pathogens become resistant to commonly used antibiotics. Therefore, vaccines developed by using recent advanced molecular techniques can be considered as an effective way of treating disease-causing pathogens in aquatic organisms (Mondal and Thomas, 2022).

As per Lillehaug, Atle. (1997) vaccines against Vibrio infections can also be administered successfully by immersion. However, due to lower levels of immunity, the need for a booster vaccination is greater when such a method is used. As regards vaccines against furunculosis, adjuvants are needed in order to achieve good protection, and, consequently, administration by injection is the only option. One of the ways to prevent diseases is vaccination and summarize important pathogens in cage culture and develop vaccines as an alternative to antibiotics to protect them.

A review on the recent advances and application of vaccines against fish pathogens in aquaculture was done by Mondal and Thomas (2022). Ma et al. (2019) and Horzinek et al. (1997) reported that vaccination has become one of the most cost-efective as well as sustainable methods for controlling several infectious fish diseases.

Stocking of more resistant large-sized fish in cages also contributes to preventing the loss of fish due to diseases. Regular disease monitor must and is important to be strengthened and continuously undertaken to monitor disease outbreaks. Moreover, selective breeding improves both growth and resistance to diseases. Cage farm facilities may require specific disease management, with emphasis on minimizing the stress in fish, transfer of disease to wild fish, and vice versa. This will reduce re-infection or infestation.

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Parasitic Diseases and their management in mariculture: A pilot study along the West Coast of India

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In recent years cage cultured marine finfish contributes significantly to global aquaculture production. FAO (2018) recorded 28.7 million tonnes (USD 67.4 billion) of food fish production from mariculture and coastal aquaculture combined in 2016. In sharp contrast to the dominance of finfish in inland aquaculture, shelled molluscs (16.9 million tonnes) constitute 58.8 percent of the combined production of marine and coastal aquaculture. Finfish (6.6 million tonnes) and crustaceans (4.8 million tonnes) together were responsible for 39.9 percent.

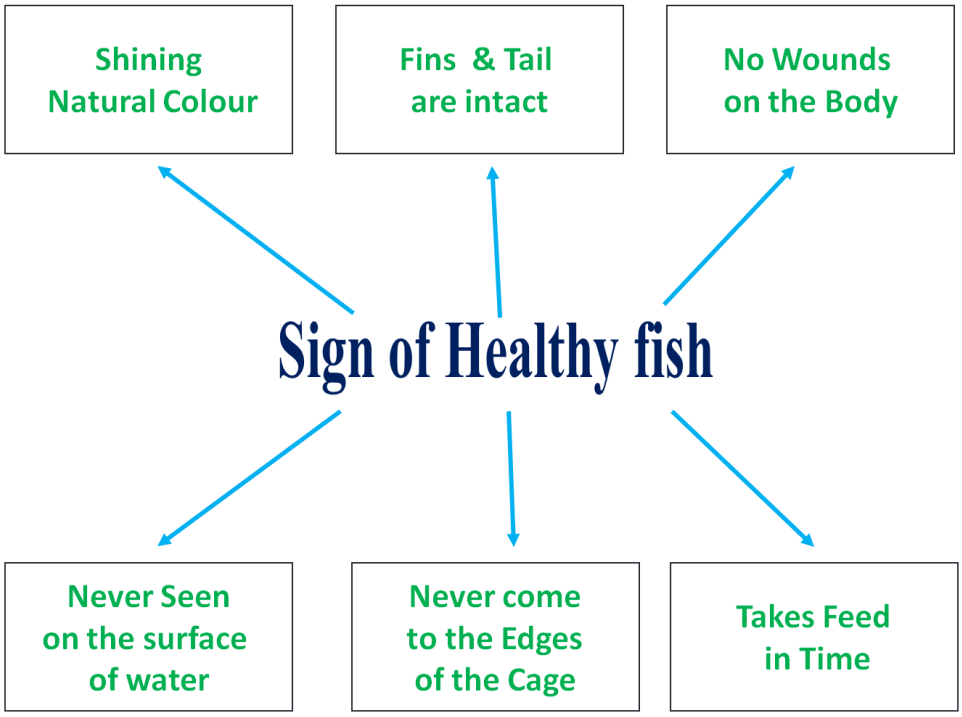
A very wide range of species are cultured worldwide, and as a consequence of this expansion in culture, incidence of parasitic infections and diseases have increased, leading to serious mortalities. Diseases in Mariculture are not only caused due to single event but are the end results of a series of linked events involving the interaction between the host, the environment and the presence of pathogens. Pathogens may include parasites, bacteria, viruses, fungi or mixture of different pathogens may cause diseases. Increasing intensification and lack of proper health management measures have lead to many disease problems. Further, the improper use of drugs and antibiotics raises concerns on the subject of human and environmental safety.

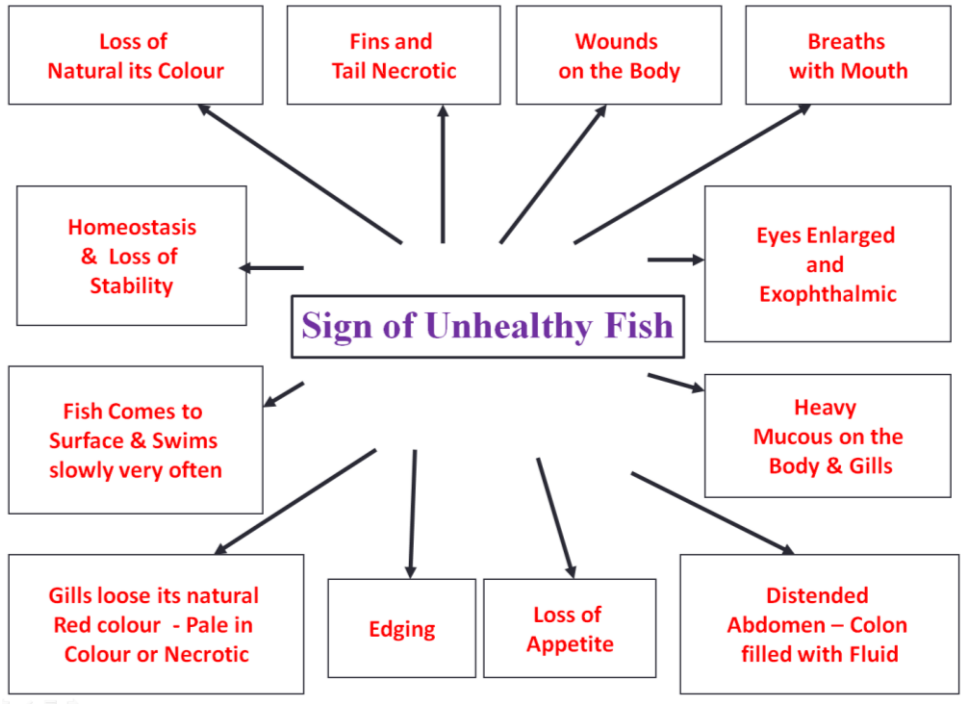
The effects of parasites on the cultured marine finfishes are still poorly understood. A large volume of information on parasitic infections in wild fish is available in India, but the significance of these as a disease entity is unclear. There is a lack of knowledge on signs of healthy fish and unhealthy fish from poor fish famer to identify disease symptoms in on time for correct disease diagnosis.

A parasitic disease in mariculture largely depends on the host species, seed (wild seeds or hatchery produced seed), feeds (wet feed or pellet), environment and management. Several reports exist on the occurrence, pathogenicity and prophylactic measures against parasitic infections in cage cultured fishes globally (Amin, O.M.,1986; Alvarez-ha et al.1992; Pellitero et al., 1993; Devaraj and Ameer Hamsa, 1977; Sobecka & Słomińska, 2007; Kumar et al.,2015; Ramudu, K.R. et al., 2017; Yin et al.,2017). A detailed study was carried out on the occurrence and

abundance of parasitic infections in cage cultured fishes along the west coast of India with special emphasis on coastal Karnataka. The following parasites were recorded from different fish species cultured in coastal Karnataka (table 1).

Here we have mentioned signs of healthy fish and signs of unhealthy fishes for easy identification of diseased and healthy fishes from culture system.



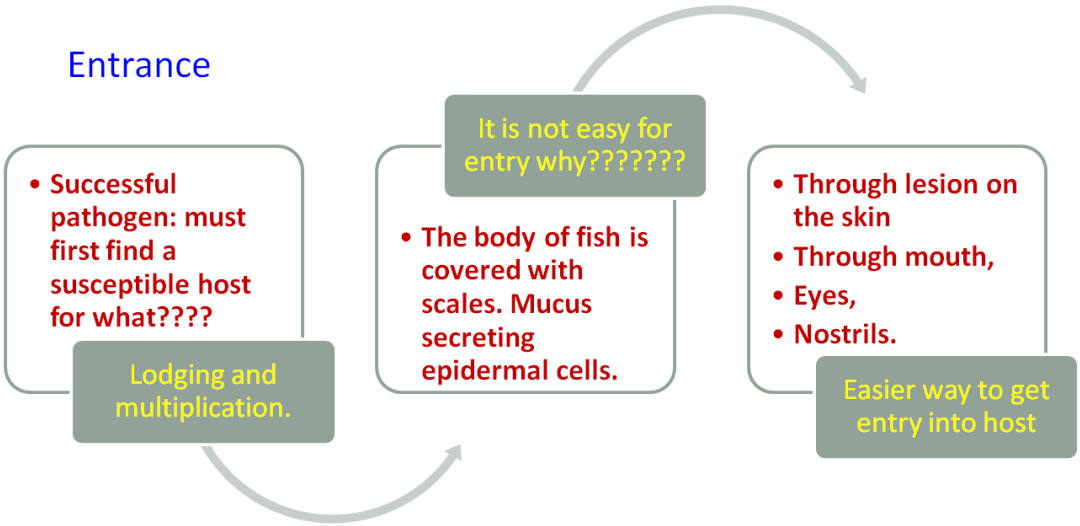


How the parasitic infestations occur?

Parasitic infections occur by following four steps.

1. Entry.
2. Establishment and Multiplication.
3. Exit.
4. Establish new infection.

Successful pathogen must first find a susceptible host for lodging and multiplication. It is not so easy, because the body of fish is covered with scales and mucus on the skin.



Entry of parasites to the culture system: Net materials, brush, scoop nets, check tray which may serve as source of disease transmission. Trash fish are commonly used as feed which can also act as a source of parasites.

Table1: Various parasites encountered in marine finfishes along the coastal Karnataka.

SN	Group	Genus/Species	Host	Target organs	Life cycle	Mode of transmission	Symptoms
1	Ciliates	*Amyloodinium ocellatum*	*Trachynotus blochii*& *Acanthopagrus berda*	Gill, Fins & Body surface	Direct	Direct contact with live dinospores	Pale gills, Dark body, High mucus production in gills
2		*Trichodina* sp.	*Trachynotus blotchii*&*Lates calcarifer*	Gill	Direct	Passive – ingestion	Rub body surface frequently against net
3	Helminth	*Dactylogyrus* sp.& *Diplectanum sp.*	*Epinephelus coioides, Acanthopagrus berda*& *Lates calcarifer*	Gill	Direct	Active – attachment	Pale gills, Lethargy, Excess mucus production
4		*Anisakis* sp.	*Lutjanus argentimaculatus*	Body cavity	Indirect	Passive – ingestion	Localized inflammation of visceral cavity

5		*Echinocephalus overstreeti*	*Acanthopagrus berda*	Intestine	Indirect	Passive – ingestion	Localized inflammation and hemorrhages of visceral cavity
6	Acanthocephalans	*Acanthocephalus* sp.	*Lates calcarifer*	Intestine	Indirect	Passive – ingestion	Mucosal tissue is damaged at the attachment site, resulting in fibroplasia, Destruction of intestinal villi, Necrotic and degenerative changes in mucosal epithelium.
7	Crustaceans	*Lernanthropus* sp.	*Trachynotus blotchii*	Gill	Direct	Active – attachment	Lethargy, Excess mucus production in gills
8		*Argulus quadristriatus*	*Lates calcarifer*	Body surface and Fins	Direct	Active – attachment	Haemorrhages, Loss of scales, Excess mucus production at affected area, Hyperemia and Hyperplasia

1. Protozoan parasites:

Amyloodinium ocellatum (Fig.1&2) &*Trichodina* sp. (Fig.3) are external ciliate parasites with a direct life cycle and were found infesting *Trachynotus blochii, Acanthopagrus berda* and *Lates calcarifer* respectively. Mixed infestation with *A. ocellatum* and *Tricodina* sp. was also encountered in *T. blochii* in nursery rearing tanks and also in brood stock. Infestation with these parasites were very high during winter season when salinities were low. Poor water quality with a high level of organic loads at the cage bottom and the high stocking density also favored infestation with parasitic protozoans. *A. ocellatum* may destroy gills and cause respiratory problems leading to mass mortalities within a short period, when the prevalence is high. Diagnosis is mainly based on microscopic wet mount study and tissue histopathological examination and though an ELISA test is existing.

Histopathology: Histopathological lesions include gill inflammation, hyperplasia and hemorrhages. Massive infections are frequently associated to mortalities, both in mariculture and sea aquaria, mainly at high temperatures.

Treatment: There no effective control measures are known for fish. Freshwater (2-4 minutes) dip treatment based on tolerance of the fish species or copper sulphate (0.75 mg/l, 12-14 days) baths have been suggested as an primary aid to control the trophonts or dinospores (Alvarez-Pellitero P., 2004). For *Trichodina* sp. (ciliatosis) can be treated with 200 mg/l formaldehyde baths for 30-60min with strong aeration or freshwater bath for one hour for three days can be applied.

2. Helminth parasites:

The most common helminth parasites encountered during the study were flukes (Monogenean & Digenean), nematodes (round worms) and trematodes (tapeworms). *Dactylogyrus* sp. (Fig.4) *Gyrodactylus* sp., *Diplectanum* sp. (Fig.5) and *Benedenia* sp. were the most common monogenean parasites recorded from marine finfish. Helminths are one of the major groups of fish parasites capable of causing serious losses in fish production. Among these parasites *Dactylogyrus* sp. and *Diplectanum* sp. appeared to be the predominant parasites recorded from Uttara Kannada district during the peak summer season. Gill flukes mainly affect gills and damage is usually done by their attachment organs as well as feeding activities. Besides direct tissue damage, the parasites also pave way for secondary bacterial infections. Infected fish shows erratic swimming behavior due to irritation and heavy mucus production on gills. In infected fish the colour of gills becomes pale and is usually covered with excessive mucus secretion.

Histopathology: Pathological changes due to Monogenean includes proliferative, degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae, telangiectasia (an indication of ballooning

dilatation in form of club deformation at the tip of secondary lamellae), congestion and fusion of secondary lamellae and mucous cells proliferation.

Treatment: Freshwater bath for 5-10 minutes or 150mg/l of hydrogen peroxide bath for 10-30 minutes can be applied to infected fishes.

Nematodes (round worms) which includes *Anisakis* sp. (Fig.6) and *Echinocephalus overstreeti* (Fig.7) were encountered in *Lutjanus argentimaculatus* and *Acanthopagrus berda* respectively during the winter season. The abundance of nematode infection was higher in winter season. Most nematodes are inhabitants of body cavities and intestine of marine finfish and are large enough to be observed by naked eye. Marine finfish can act as both definitive and intermediate hosts of nematodes. Developmental stages of nematode larvae by its feeding and migration may cause inflammation and haemorrhage in host tissues, leads to emaciation, reduced growth/production and sometimes mortalities in cultured fishes. Anisakiasis caused by the nematode larvae is a serious problem in marine fishes.

Histopathology: Pathological changes due to *Anisakis* sp. includes atrophy of intestinal mucosa, necrosis and degeneration of the intestinal tissue, causing damage to the whole thickness of the bowel wall.

Treatment: Eliminate entry of intermediate host such as copepods in to the culture system, frequently disinfects the culture system with detergent or disinfectant such as chlorine. Avoid feeding with infected trash fish.

***Acanthocephalus* sp.** : Infection with *Acanthocephalus* sp. were observed in Asian seabass, *L. calcarifer* during the spring season with a prevalence of 1-2%. The parasite causes necrotic, hemorrhagic ulcers in the intestine of the host. Damage is mainly due to the mechanical injury caused by the attachment of spiny proboscis on the intestinal wall leading to inflammation and tissue necrosis. Acanthocephalans (Fig.8) with an anterior proboscis (Fig.9) covered with numerous hooks and they feed on the intestinal walls of host.

Histopathology: histopathological changes due to *Acanthocephalus* sp. were reported by so many authors (Ferguson H, 1989 & Kim et al., 2011), such as when the presoma and the anterior part of the metasoma of *Longicollum pagrosomi* and *Acanthocephalus* sp. passed through the intestinal wall and infect the intestinal tissue, perforating the loose connective tissue. In the inflammatory connective tissue, collagen and muscle fibers were fragmented and revealed fractional necrosis. Lipid drops and eosinophilic granular cells aggregated in the connective tissue of the tissue capsule.

Treatment: Eliminate entry of intermediate host (copepods) in to the marine farms, frequently disinfects the marine farms with detergent or disinfectant such as chlorine and avoid feeding with infected trash fish.

Crustacean parasites:

The main groups parasitic crustaceans are Copepoda and Isopoda. Among Copepoda, species of the genera *Argulus, Ergasilus, Caligus, Lernanthropus, Lerneaocera, Lernaea* and *Lepeophtheirus* (sea lice) causing infestation in different marine and freshwater fish. *Argulus quadristriatus* (Fig.10) was the most common crustacean parasitic infestation found in Asian seabass, *L. calcarifer* with a prevalence of 7-50% during summer months. Infestations with *Lernanthropus* sp. (Fig.11 & 12) was also recorded in *Trachynotus blotchii* during summer. The first symptom is an abnormal behaviour of fishes. They rub against net (flashing) and jump out of the water. Anorexia and hyperproduction of skin mucus are also observed. Gill damage (leading to respiration problems), inflamed wounds, ulcers and excess mucous production can be due to crustacean bites.

Histopathology: Histopathological changes in infected fish by copepod are epithelial erosions, ulceration around the site of attachment of the parasite's mouth organs, hemorrhages and around the penetration sites of the claws there occur tissue necrosis, proliferation happens around the site of penetration of antennae (Noga EJ., 2000). Some copepods may be deeply embedded within the skin and elicit host response, mainly localized in mild dermal fibrosis and epidermal hyperplasia (Ferguson H, 1989).

Treatment: Fresh water bath for 10-15minutes or 200-250 mg/l formalin bath for 1hour. One of the major problems in implementing control measures to prevent parasitic infection in cage culture in India is the continuation of cage culture throughout the year. There are no breaks in the culture cycle (like crop holiday), before the next batch of fish is stocked. As a consequence, new fish introduced into the farm would likely be infected with one or more species of parasite which already exist on the farm. Therefore, the fish farm itself is a reservoir for parasites.

Marine leeches:

Leeches are the important fish pathogens in the phylum Annelida. Both fresh water and marine leeches have worldwide distribution. Leeches are a different group of organisms with many species living in marine environments, including the deep ocean and intertidal regions. They can be predators or temporary ectoparasites and feed on host, this diversity makes them an interesting group of animals to study. At present study in Uttara Kannada district Asian seabass, *L. calcarifer* infected with marine leeches with a prevalence of 50% during winter months.

Leeches can potentially affect the health of fishes in a different ways. Leeches alone are generally not considered important fish pathogens. Effects are usually confined to a small area and limited to attachment or feeding sites on the skin, fins, gills or mouth. The muscular caudal sucker used for attachment and it is usually causes little damage; however, leeches that are semi-permanent parasites may elicit a substantial host tissue response at the

attachment site. Rhynchobdellid leeches feed on host blood or tissue fluid by means of a protrusible proboscis, which is inserted into host tissue and this feeding activity may produce localized petechial haemorrhage (Sloan et al., 1984; Jones and Woo, 1990).

Leeches can be difficult to collect and to identify. They are usually sufficiently large to be detected by the naked eye and occur on the body surface, fins, gill cavity and mouth. For correct identification it is important to observe leeches alive and to note as many external characters as possible. Leeches should preferably be removed from the fish and then fix it, especially for species identification if it is desired. Leech infestation can also be diagnosed by histopathology.

Histopathology: Even though pathology is usually localized, heavy infestations can result in severe epidermal erosion and even mortality because of large amounts of blood loss or secondary effects of multiple feeding wounds. Leeches by themselves are only rarely associated with serious pathological effects. Pathology may depend on the relative size of the leech compared with the fish. Kabata (1985). They often cause very localized histopathological changes, including cellular infiltration, erosion of the integument under the attachment site and hyperplasia of the epidermis around the caudal sucker. Localized subcutaneous haemorrhages often occur at leech feeding sites. Paperna and Zwerner (1974) reported removing over 500 *M. lugubris* from a single moribund white catfish, *Ictalurus catus*, in the York River estuary in Virginia, USA. Extensive histopathological changes caused by the leech included inflammation, displacement and erosion of the dermis and hyperplasia of the epithelium (Noga et al., 1990). In addition to anaemia, secondary bacterial infection can also develop in the ulcers and the leeches may transmit blood flagellates.

Treatment:

Treatment: Manual removal using cloth, 200-250mg/l formalin bath for 1 h. or A 50 ppm formalin bath for 1 h was effective in controlling *Z. arugamensis* on orange-spotted grouper in the Philippines (Cruz-Lacierda et al., 2000). Freshwater baths may also be effective for marine leeches if the fish host can tolerate the bath. Prevention is the best control and, according to Bauer et al. (1973). Culture facilities must be cleaned with chlorine and exposed to intense sunlight for several weeks prior to stock the seeds to eliminate cocoons of the parasites.

Conclusion:

A successful parasitic diseases control programme consists of the selection of healthy fish, preventive measures, good management practices, quarantine, correct analysis and, if essential, therapeutic treatment. Unfortunately, under farming conditions in the India, farmers often lack of technical expertise in proper health management at local area of farming conditions. An experienced farmer might know that the fish are not well but may not know the cause and what needs to be done. Technical support for accurate diagnosis and for recommending appropriate treatments is generally lacking in local area where mariculture activity is going on. Recently ICAR-CMFRI has started giving technical support to the fish farmers who is doing

marine cage culture throughout the coastal states in India. Regular health monitoring and early diagnosis is a key for control of parasitic diseases. When unusual symptoms are first observed in a particular fish cage, actions must be taken quickly to reduce or eliminate the number of parasites in culture system.

Fig.1. *Amyloodinium ocellatum* (50x)

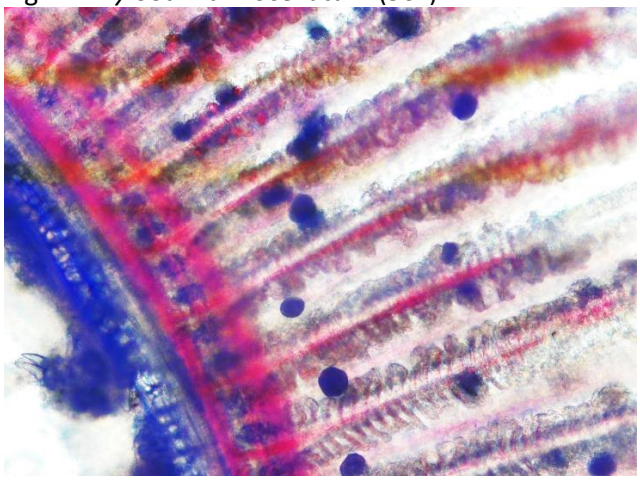


Fig.2. *Amyloodinium ocellatum* (Histo pathology section)

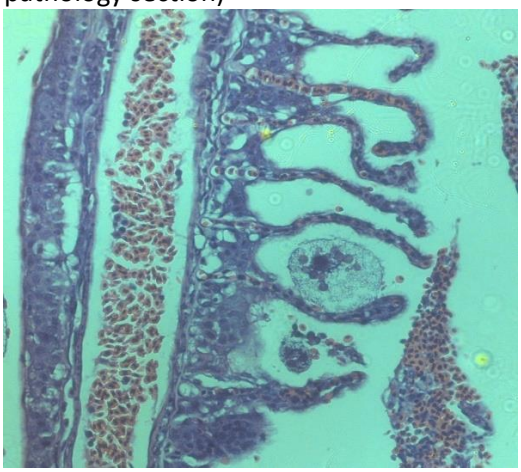


Fig.3. *Trichodina* sp. (400x)

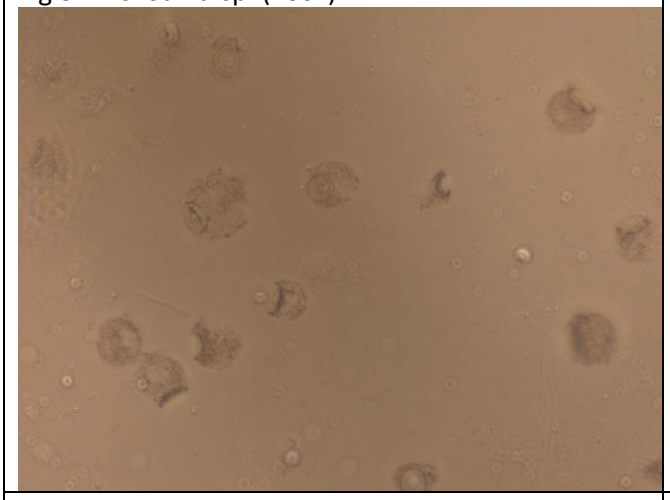


Fig.4. *Dactylogyrus* sp.

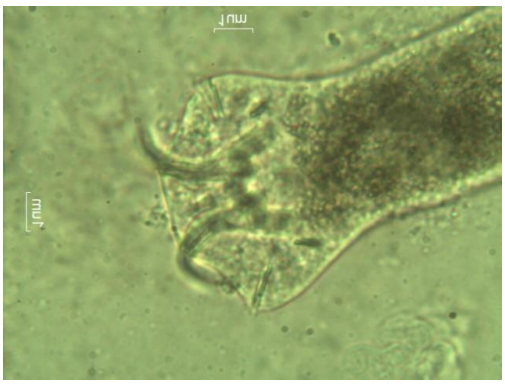


Fig.5. *Deplictanum* sp.

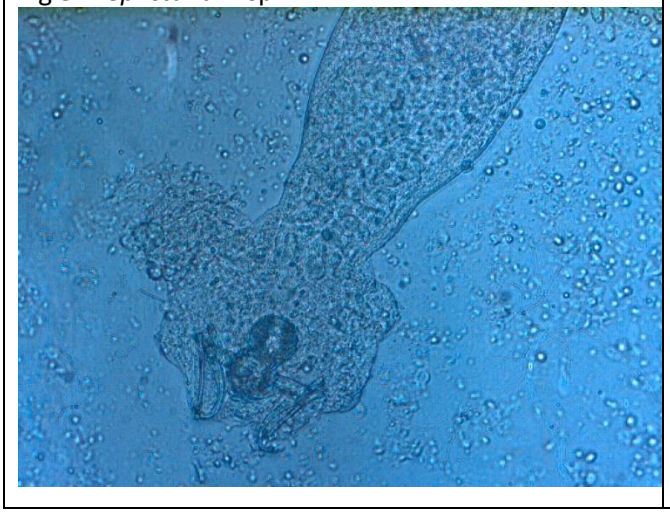


Fig.6. *Anisakiasis* sp.

Fig.7. *Echinocephalus overstreeti* from gut

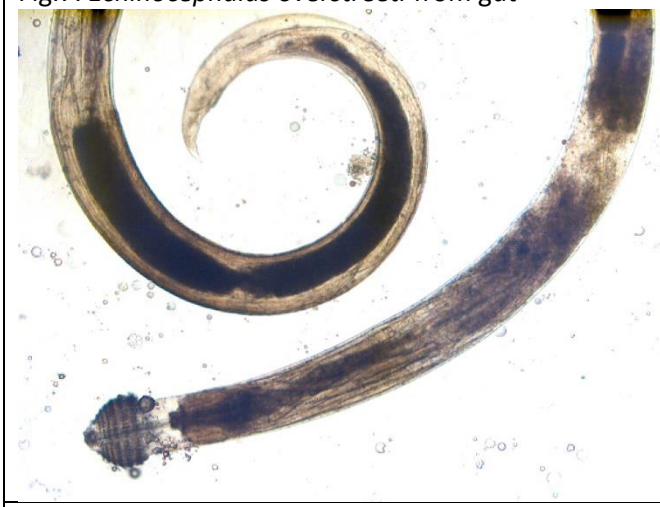


Fig.8. *Acanthocephalus* sp.

Fig.9. The proboscis of *Acanthocephalus* sp.

Fig.10. *Argulus quadristriatus*

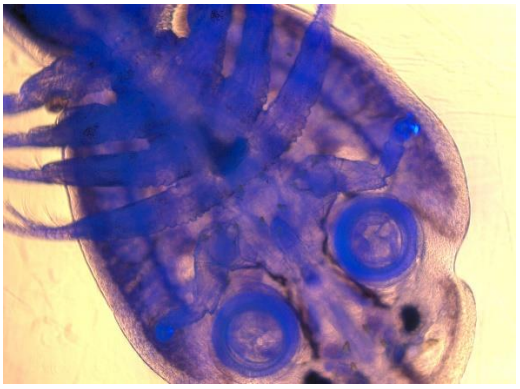


Fig.11. *Lernanthropus* sp. on the gill

Fig.12. *Lernanthropus* sp.

Good management Practices (GMP) for parasitic infestation:

- Fish fry / fingerlings must be screened and quarantined before stocking into the cages, grading of fish must be done at regularly (within the first 3-4 months of stocking).
- Fish health monitoring during the culture period must be carried out continuously at regular intervals.
- Prophylactic treatment: If the parasitic disease outbreak occurs, freshwater treatment should be carried out for three days, on the first one, followed by a third and one fifth day.
- Immersing fish for 10 - 30 minutes in freshwater mixed with formalin (100-200 ppm), it should be depending on the tolerance of the fish species and their size.
- Treatment should be during the coolest part of the day.
- Various disinfectants such as chloramine-T and formalin, hydrogen peroxide, anti helminthics (such as praziquantel and fenbendazole), organophosphates (such as dichlorvos), pyrethroids (such as deltamethrin), avermectins (such as ivermectin) and chitin synthesis inhibitors (such as diflubenzuron) has been tested and can be used against fish parasites.
- Keep on monitor during the treatment and be prepared to stop treatment immediately if adverse reactions like gasping for air, strange swimming behaviour, etc.
- Regular net exchange of cages and disinfection of nets must be done using bleaching powder or formic acid (3%) in order to eliminate the attachment of fouling and parasitic organisms.
- Cage structure and nets must be checked at regular intervals to avoid physical injuries to the fish.
- Entry of visitors may be restricted into the culture system to avoid contamination through net materials, brush, scoop nets, check tray which may serve as source of disease transmission.
- Optimum stocking densities should be maintained in cages to reduce the stress.
- Usage of excessive feed in cage culture may lead to the development of high organic load at the bottom of the cages, favouring diseases. Hence usage of excessive feed must be avoided.

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Farm Business Planning and Budgeting for Small-scale Mariculture Enterprises

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Introduction

Mariculture and allied enterprises have immense scope to support the livelihoods of small holders, especially the resource-poor coastal dwellers. There are wide variety of enterprises in the sector such as cage culture farms, ornamental fish farms, fish hatcheries and seed production units, mussel and oyster culture farms, clam processing units, pre-processing units and fish value added products units. Like any other small-holder enterprises, success of such units depends considerably on farm planning, budgeting, accounting as well as market analysis and business development. This chapter deals with the basic accounting and economic principles and practices based on which a successful mariculture-based enterprise can be operated.

Farm business planning

Farm business planning is integral to farming as it involves adoption of business methods in every phase of farm activity. It is an integrated, co-ordinated and advance programme of actions which seek to present and opportunity to farmers to improve their level of income. A detailed farm business plan should show the enterprises to be taken up and the various practices to be followed in production, use of labour, investments to be made and other details. It enables the farmer to achieve his objective (profit maximization or cost minimization) in a more organized manner. As in crop farming, farm business planning is an important activity in fishery related enterprises as well. The following are the major rationale of farm business planning:

- Farm business planning is a necessary pre-requisite for running a profitable enterprise.
- Helps in systematic assessment of the resources in hand and schedule farm production accordingly.
- Guide to better management and future decisions.
- Helps in mid-term corrections.
- Helps in assessing production and marketing decisions.
- Aids in inventory management.
- Helps in keeping track of income and expenditure thus ensuring profitability.

Types of farm plans

a. **Simple farm plan:** It is adopted either for a part of the farm or for one enterprise or to substitute one resource with another. It is relative easy to implement. Changes in farm activities are generally initiated with such simple farm plans.

b. Complete or whole farm planning: This involves planning for the whole farm. Such planning is adopted either at the beginning of farming or when major changes are contemplated in existing organization of farm business.

Main elements of farm business planning

The main elements of farm business planning are the following:

- Production analysis
- Resources at hand
- What is the scale of production and product mix?
- Quantity of inputs needed
- Source of inputs and supplies
- Technology available and source
- Where is the market?
- Harvest scheduling
- Marketing strategy
- Preparation of enterprise budget - costs and earnings estimates
- Record keeping
- Cash flow and accounting
- Farm inventory analysis
- Farm efficiency measures

Farm budgeting

Budget is essentially a presentation of costs and returns accompanied by a statement showing the physical quantities of inputs and output associated with each value figure. The objective of drawing up a budget is to measure the returns expected from the plan. Farm budgeting is a method of analyzing plans for the use of resources at the command of the decision maker. In nutshell, the expression of farm plan in monetary terms by estimation of receipts, expenses, and net income is called budgeting.

The three common objectives of farm budgeting are:

a. To estimate the profitability of a particular pattern of organization.
b. To determine the change in profitsthat are likely to follow a particular change in organization, and
c. To compare different organizational patterns or alternative changes in organization on a profit basis.

Type of farm budgeting

a. **Partial budgeting (Enterprise budgeting):** It refers to estimating the outcome or returns for a part of business i.e., one or few activities. In situation where relatively small modifications have to be made to existing organization, a partial budget will suffice.
b. **Complete budgeting:** This method is used to make out a plan for the whole farm. In situations involving extensive remodeling of the farm organization, a full budget is called for. This entails setting out all the individual costs and return items for the farm, so that overall net return is from the whole unit.

A typical example for an enterprise budget for small mariculture unit is presented below.

Capital cost			
Cage 10m X 5m with GI frame	80,000	10	800000
Total A			800,000
B. Operational Cost			
Asian Seabass seed	10000	40	400000
Pearl spot seed	2000	10	20000
Seabass feed			1344000
Pearl spot feed			20000
TOTAL B			1784000
C. COST-BENEFIT ANALYSIS			
C1. Annual fixed cost			
a. Depreciation on capital investment, @20%			160000
b. Insurance premium @ 2% of the capital investment			16000
c. Interest on 75% of the capital investment @12% per annum			72000
d. Administrative/Other expenses @ 1% of 75% capital investment			6000
Total Annual fixed cost C1 (a+b+c+d)			254000
C2. Annual Variable Cost			
a. Annual operational cost (B)			1784000
b. Interest on operational cost @ 12%			214080
TOTAL Annual variable cost C2 (a+b)			1998080
TOTAL OPERATIONAL COST (C1+C2)			2252080
D. INCOME			
a. Income from Sea bass production (6400 Kg @Rs.500)			3200000
b. Income from Pearl spot production (240 Kg @Rs.600)			144000
TOTAL INCOME			3200000
FINANCIAL ANALYSIS			
a. Operating cost			2252080
c. Total Cost			2534054
d. Gross Revenue			3200000
e. Net Operating income			947920
f. Net profit			693920
BC ratio			1.26

Estimation of depreciation

Depreciation is a method of reallocating the cost of a tangible asset over its useful life span. In other words, it re-estimates the value of a fixed asset every year taking account of its loss value due to wear and tear. It is worked as per the following example:

Cost of cage = Rs. 60,000/-

Scrap value = Rs. 5000/-

Life time of machine = 10 years

Depreciation = (60,000 – 5000)/10
= 55000/10
= Rs. 5500

Cost concepts

Cost concept approach to farm costing is used widely in India. The three cost concepts in brief, are Cost-A1; Cost-A2 and Cost C. The different cost items that are to be included under each cost concept are detailed below.

Cost A1

- Casual hired labour
- Hired machine labour
- Imputed value of own machine labour
- Feed
- Chemicals
- Seeds
- Maintenance charges
- Interest on working capital
- Depreciation

Cost A2 = Cost A1 + Rent paid for leased in land

Cost B = Cost A2 + Imputed value of owned land + Interest on owned fixed capital

Cost C = Cost B + Imputed value of family labour

Farm record keeping

Farm record keeping is a system of records written to furnish a history of business transactions with special reference to its financial side. The objective of farm records and accounts is to provide control over business and improve the management of farm.

Type of farm records

- Physical farm records
- Financial farm records
- Supplementary farm records

Physical records

- Farm map
- Land utilization records
- Production and disposal record for crops/livestock/fishery
- Labour records
- Machinery records
- Feed records
- Stock and store register

Financial records

- Farm inventory
- Cash flow statement
- Capital asset and sale register
- Cash sale register
- Credit register
- Purchase register
- Wage register

Supplementary records

- Auction register
- Hire register

- Stationary register

Cash flow statement

Cash flow statement provides the details of receipts and payments. It is prepared for a specific period. It reflects net changes in cash balance and helps to capture the progress of farm business systematically. A hypothetical example for a typical cash flow statement in a small fish farm is presented below:

Cash flow statement: An example

Date	Particulars	Expenditure	Income
1-1-2018	Purchase of seeds	10,000	
5-1-2018	Purchase of feed	15,000	
10-1-2018	Sales revenue on fish		26500
12-1-2018	Purchase of farm implements	21200	
15-1-2018	Wage payment	25,000	
25-1-2018	Rent on leased-in land	4,500	
28-1-2018	Proceeds from sale of seeds/fingerlings		65,000
	Total for January, 2018	75700	91500
1-1-2018	Opening balance	24500	
31-1-2018	Closing balance	40,300	
	Net change in cash		15800

Balance sheet/ Inventory statement

Balance sheet is a statement of physical properties pertaining to a farm business in terms of assets and liabilities. Assets and liabilities include fixed/ working/current. Contrary to cash flow statement, it is prepared for a point in time. Balance sheet essentially reflects net changes in inventory. It helps to capture the financial health and stability of the business. A hypothetical example for a typical balance sheet in the context of a small fish farm is presented below:

Balance sheet for a fish farm: An example

Liabilities (Rs)	**Value (Rs)**	**Assets (Rs)**	**Value (Rs)**
Current liabilities		Current assets	
Fertilizers	5,500	Cash in bank	25000
Feeds	8,500	Cash in hand	15,000
ST loan	45,000	Grains stored	20,000
working liabilities		working assets	
MT loan on equipment	75,000	Machinery & equipment	1,50000
Insurance payments	25,000	Standing fish stock	35,000
Fixed liabilities		Fixed assets	
		Land	10,00,000
		Farm sheds	2,50,000
Total liabilities	1,59,000	Total assets	14,95,000

Net worth	13,36,000

Farm efficiency measures

An important element in farm business management or decision making relates to the manner in which available resources are allocated. A measuring indicator is necessary to provide guides and standards for appraising accuracy of decisions regarding the use of resources. There are two broad types of efficiency measures, viz., physical efficiency measures and value efficiency measures. They can be further categorized into aggregate or absolute measures and ratio measures. Some of the farm efficiency measures widely used in the context of farm business planning are presented below:

Aggregate measures:

- Gross income = Value of main product + by-products
- Net operational income= Gross income – total operational cost
- Net profit = Gross income – (total fixed costs + total operational costs)
- Net worth = Total assets - total liabilities

Ratio measures:

- Cropping intensity = 100*(Gross farmed area / Net farmed area)
- Benefit-cost ratio (B:C Ratio) = (Present value of gross benefits)/(present value of total cost)
- Break-even point = The point where total costs equals total revenue
  = Fixed costs/(Price per unit- operational cost)
- Capital turnover rate = 100* (Gross income/Total value of farm assets)

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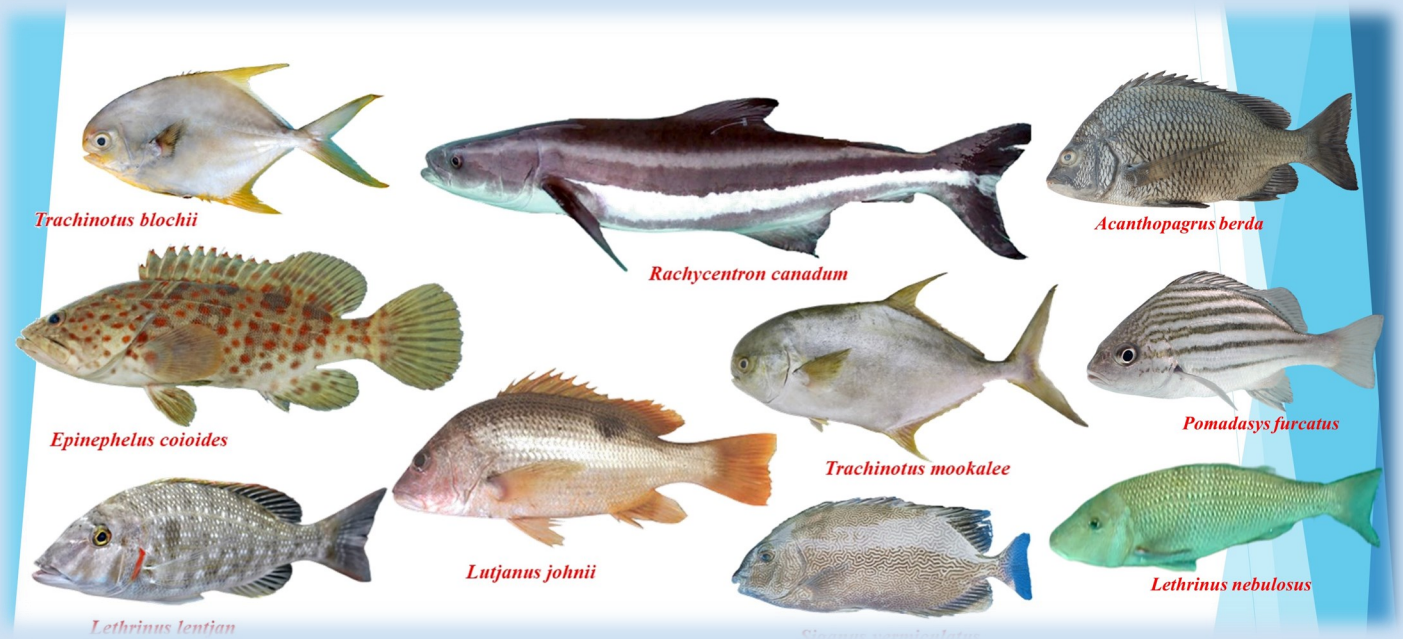
Notes

List of Officers, Department of Fisheries, Govt. of Kerala who attended training programme

Sl. No.	Name	Designation
1	Shri. Benson K.	Asst. Director of Fisheries
2	Smt. Neetha Susan David	Fisheries Extension Officer
3	Smt. Fathima S. Hameed	Fisheries Extension Officer
4	Smt. Merlin Alex	Fisheries Extension Officer
5	Smt. Saritha K. V.	Asst. Fisheries Extension Officer
6	Smt. Ramya K. D.	Asst. Fisheries Extension Officer
7	Smt. Reshmi P. Rajan	Asst. Fisheries Extension Officer
8	Shri. George N. S.	Asst. Fisheries Extension Officer
9	Dr. Sini Wilson	Fisheries Officer
10	Shri. Asif A. S.	Fisheries Officer



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