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Training manual on
Farming and seed production technology of brackishwater fishes
18 – 23 December, 2021

ICAR - CENTRAL INSTITUTE OF BRACKISHWATER AQUACULTURE
KAKDWIP RESEARCH CENTRE
Kakdwip, South 24 Parganas, West Bengal - 743347

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on

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FOREWORD

Aquaculture sector has been conventionally playing a pivotal role in addressing the key global challenges of food and nutritional security, poverty alleviation and economic development of billions of people. Since its establishment in 1987, ICAR-CIBA has been playing a highly constructive role in development of brackishwater aquaculture in India through the development of viable technologies on seed production, farming, feeds and disease diagnosis of shrimp, fish, and crab. Due to diversified nature of brackishwater aquaculture and species diversification it provides many options to farmers to choose suitable species for farming. Availability of quality fish seed in adequate quantity is important to expand the brackishwater finfish culture in different culture systems *viz.* pond, RAS, cage etc. CIBA has developed comprehensive technology packages for seed production of seabass (*Lates calcarifer*), milkfish (*Chanos chanos*), pearl spot (*Etroplus suratensis*), catfish (*Mystus gulio*) and brackishwater ornamental fishes such as spotted scat (*Scatophagus argus*) and silver moony (*Monodactylus argenteus*) and orange chromide (*Etroplus maculatus*). Apart from this, ICAR-CIBA has also developed and successfully demonstrated farming methods of brackishwater fishes, *viz.* polyculture, multi-tier farming, biofloc based farming, periphyton based farming, IMTA etc. among farmers. These technology developed by ICAR-CIBA will pave the way forward for development of brackishwater fish farming in our country. ICAR-CIBA also takes a lead in organising routine need-based training programmes to train personnel in all aspects of brackishwater aquaculture. During this year, a training programme on "**Farming and seed production technology of brackishwater fishes**" is being organized for 6 days from 18th December to 23rd December, 2021 at its Kakdwip Research Centre. Aim of the current training programme is to train region-specific brackishwater farmers, entrepreneurs and officials from state fisheries department on captive breeding and farming of brackishwater fishes. This special publication covered the seed production and farming technologies of brackishwater fishes.

I hereby congratulate the entire team and wish the training programme a grand success.

(K.P. JITHENDRAN)

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Overview of brackishwater aquaculture in India

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1. Introduction

Aquaculture is one of the fastest-growing industries in the World (Tacon, 2020) and it has important role in the economic development, food and nutritional security, national income, employment opportunities (Kumar and Shivani, 2014). It is an important source of animal protein for billions of people and capture fishery and aquaculture serves the livelihoods of more than 10% of the global population (Anonymous, 2020). Aquaculture supplies not only dietary nutrient for human consumption, but also provides opportunities for employment and income generation in the rural areas (Jayasankar, 2018). India is the second most populous country in the world with a total population of 1.21 billion. The greatest challenge the country faces is to ensure food security of the largely undernourished protein starved population in rural as well as urban areas, especially in the context of declining land resources available for agriculture and animal husbandry (Mukherjee and Chakraborty, 2010). Carps in freshwater and shrimps in brackishwater form the major areas of activity. About 40% of the available 2.36 million hectares of freshwater resources and 13% of a total potential brackishwater resource of 1.24 million hectares is under use at present (FAO, 2014). In India, brackishwater aquaculture cover the vast 1.2 million ha coastal and 8 million ha of inland salt affected areas, and has a potential to contribute significantly to aquaculture production. Brackishwater farming in India is an age-old traditional system confined mainly to the '*bheries*' (manmade impoundments in coastal wetlands) of West Bengal, '*gheris*' in Odisha,'*pokkali*' (salt resistant deep water paddy) fields in Kerala, '*khar lands*' in Karnataka and '*khazans*' in Goa coasts. These systems have been sustaining production of 500–750 kg/ha/year with shrimp contributing 20-25% with no additional input, except that of trapping the naturally bred juvenile fish and shrimp seed during tidal influx (FAO, 2014). The boom period of commercial-scale shrimp culture started in 1990 and the bust came in 1995-96, with the outbreak of viral disease (Mehta, 2009). Development of bio-secured shrimp farming technology and better management practices, shrimp farming started to regain its lost glory

during early years of this century. This bio-secured shrimp farming involves no water exchange, pond and pond water disinfection, use disease free hatchery produced seeds, feeding management and adoption of best management practice. Further to ensure sustainability of brackishwater aquaculture, diversification of culture systems involving various species could be a practical approach. Since its establishment in 1987, ICAR-CIBA has developed several innovative aquaculture technologies, which have been widely adopted by the farmers and entrepreneurs for sustainable development of brackishwater aquaculture.

2. Development of brackishwater aquaculture

In early 1911, with the suggestion of James Hornell salt water fish farming was developed in Madras Presidency which led to establishment of marine fish farm near Tuticorin, Tamil Nadu. This farm was initially stocked with mullets and sand whiting (*Mugil* spp. and *Sillago* sp.). During 1940-42 establishment of Narrakal fish farm at Kochi, symbolized the brackishwater fish farming (mullets and milk fish). Thereafter, development of Ayiramthengu fish farm near Kayamkulam lake in Kerala was an important step in the development of the culture of brackishwater fishes, pearlspot, mullets and milkfish (Tampi, 1958). Initiation of All India Coordinated Project (AICRP) on brackishwater fish farming by ICAR in 1973 resulted in the development and establishment of many fish and shrimp farming technologies. In the early 1990's a boom in shrimp farming activities faced a set-back due to the disease outbreak (white spot syndrome virus, WSSV) which continues to pose a major challenge to the shrimp farming sector even today. India, the seventh-largest economy in the world, is the second largest producer of fish and shellfishes from aquaculture. Since its establishment of ICAR-CIBA in 1987, this institute has developed viable technologies on seed production, farming, nutrition, disease diagnosis and management of shrimp, fish, and crab.

3. Farming of shrimp, crab and fish in brackishwater

3.1 Seed production and farming of crustaceans

3.1.1 Penaeus indicus

Indian white shrimp, *Penaeus indicus* has been a high-valued commercial species in Indian waters. It has been widely fished throughout the Indo- Pacific, and aquaculture potential of this species has been well recognized as early as 1970s. In India, hatchery technology and initial trials on the domestication was carried out before 1980s (Muthu *et al.,* 1992). However, when shrimp farming has become popularized in 1990s, the priority of this

species has been overlooked and attention has been shifted to giant tiger shrimp, *P. monodon,* possibly due to the farmers' preference on the success of south Asian model of shrimp farming development (Muthu et *al.,* 1992).

Life cycle of *P. indicus* is similar to that of other penaeid shrimp. The adults live, mature and breed in the sea, larval development takes place in the sea and post larvae migrate to the estuaries and coastal lagoons. After few months of development, sub-adult migrates to sea. ICAR-CMFRI has worked on development of small and medium scale hatchery technology for the *P. indicus.* Recently, CIBA has widely demonstrated the *P. indicus* farming in different coastal states of India (CIBA, 2016). This species is an ideal species for growth improvement through selective breeding and could be an alternative for the exotic American shrimp, *P. vannamei.*

Potential advantages of developing selectively bred *P. indicus* are:

- *P. indicus* is a native species therefore all the measures to import *P. vannamei* could be avoided or minimized.
- *P. indicus* is not a natural host of many emerging diseases, and it is comparatively easy to develop disease free stock.
- Due to availability of distinct genetic populations of *P. indicus* it has potential for genetic improvement.
- *P. indicus* is native to India it may exhibit greater tolerance and better growth than *P. vannamei* in India
- Due to wide salinity tolerance/ strong osmoregulation, it can be farmed under wide range of salinity conditions.

3.1.2. Penaeus merguinesis

Banana shrimp, *P. merguinesis,* is a potential species for aquaculture in India and several Asian countries. Morphology of this species is similar to *P. indicus,* however, it grows larger than *P. indicus.* The commercial availability of this species is in North West to the Karwar coast and to the Odisha coast north of Chilka lagoon. Life cycle of *P. merguinesis* is similar to *P. indicus.* Hatchery technology of this species has been standardized by CIBA, and this species can reach full ovarian maturation under captivity. Further it can mature and spawn without eye stalk ablation. Thus, hatchery production could be independent from the wild stock, indicating the potential for the domestication and selective breeding program.

3.1.3. Penaeus japonicus

Penaeus japonicas is commonly known as Kuruma shrimp. This is the first penaeid species whose life cycle has successfully closed. It is one of the most costly and luxurious food item in most of the top Japanese restaurant s. This species is widely distributed in Indo-west Pacific from the coast of east Africa and red se to Fiji and japan. In India, it is found in stray catches except in Maharashtra where there is a minor ·seasonal fishery during June to September. Although the species has a typical life history of penaied, the migration of post larvae to less saline estuarine habitat is unlikely (Muthu *et al.,* 1992). ICAR-CIBA has developed a successful hatchery technology for *P. japonicus,* and the species was successfully domesticated. Successful genetic improvement program of this species has been achieved in Australia and Japan.

3.1.4. Mud crab

Among the four commercially important mud crab species, only two species are reported from Indian waters: *Scylla serrata* and *S. olivacea* (Balasubramanian *et al.,* 2014). *S. serrata* are the largest species, commonly known as green crab whereas *S. olivaceae* has orange to reddish colouration in claws and carapace hence known as orange crab. The maximum size of *S. serrata* so far recorded in the wild is 240 mm (carapace width) and 2. 8 kg (body weight) and *S. olivacea* is 181 mm (carapace width) and 0.83 kg (body weight). Mud crab possesses all the character for a viable aquaculture, like high market price, rapid growth, simple feed, and less stringent environmental requirements. *Scylla serrata* is closely linked to the estuarine and mangrove habitats, and market size and reproductive females are frequently obtained from these habitats. However, the early larval phase has not been recorded from the estuarine mangrove habitats. Hatchery production of *Scylla* has been standardized recently although the survival at the hatchery phase is low (Balasubramanian *et al.,* 2013).

Aquaculture of this species needs long rearing period (10-12 months; below 1 g to above 500 g). This long culture period hinders the production efficiency, survival rate, and moreover, farmers are reluctant to adopt this farming as it take long period for obtaining revenue. In order to circumvent these issues and optimize the economy, a three tire modular farming system (multi- phased culture system), comprising a three months nursery rearing, and four months of mid grow-out and three months of final grow-out system, has been developed by CIBA (CIBA, 2015). Forty six percent of juveniles were survived after

three month s of rearing with an average body weight of 84.8 g and 280 kg/ha production. In the mid grow-out phase, nursery reared juveniles are reared at a stocking density 0.1 crabs per sq. m and reared for three months. The harvest weight was 270 g with a production of 1110 kg/ha (Balasubramanian *et al.,* 2013). The final grow-out was for three months with a very low stocking density (0.01 crabs/ m^2) with 80% survival and a production of 1168 kg/ha. Mud crab is also found to be better option for polyculture with finfishes, and productivity of the mud crab in polyculture pond is about 2500 kg/ha. Some farmers are practicing crab fattening in the coastal districts of West Bengal with considerable success. Survival of 70-80% is generally achieved after 20-30 days of fattening operation (Suseelan, 1996). With the intervention of ICAR-CIBA, some progressive farmers in Kakdwip and Namkhana block, West Bengal have started crab monoculture with 1-2 ton/ha production with wild seed of *Scylla olivacea* and hatchery produced seeds of *S. serratae*, in polyculture, some compatible fish like, *Mystus gulio*, *Liza parsia* and *Mugil cephalus* are also co-cultured with crab to earn additional profit. Recently, CIBA has developed the multi-tier farming technology of crab and brackishwater fish by adopting co-culture of crab in box and fish in net cage and open water (Christina et al., 2020).

3.2 Finfish aquaculture

3.2.1 *Seed production technology*

The commercially important brackishwater food fish species are Asian seabass *Lates calcarifer* (Bloch, 1790), grey mullet *Mugil cephalus* (Linnaeus, 1758), milkfish, *Chanos chanos* (Forsskal, 1775), pearlspot, *Etroplus suratensis* (Bloch 1790) and *Mystus gulio* (Hamilton, 1822). In the year 1997, a significant milestone achieved with respect to brackishwater finfish aquaculture in our country was the successful breeding of Asian seabass in captivity at the Central Institute of Brackishwater Aquaculture (Thirunavukkarasu *et al.,* 1997; 2001; 2004) which in the course of events led to the establishment of the first brackishwater/ marine finfish hatchery of our country located at CIBA, Chennai. The controlled breeding and seed production of cobia, (*Rachycentron canadum*) was developed by CMFRI and CIBA (2012-13). Recently, CIBA has developed captive breeding technology of milkfish, *Chanos chanos* (CIBA Annual Report, 2015-16). In addition to this an avenue has come by successful captive breeding and seed production technology of spotted scat (*Scatophagus argus*) (CIBA Annual Report 2011-12), pearl spot (*Etroplus suratensis*) (Sukumaran et al., 2017) and brackishwater catfish, *Mystus gulio* (Kumar et al., 2018).

3.2.2. *Farming of seabass*

The seed production technology developed by CIBA has already been commercialized and the feed technology (CIBA Bhetki AHAAR) is ready for commercialization. Cage culture of seabass has been carried out on an experimental basis by different research organisations in open seas or pond systems. Scientific farming of brackishwater finfishes is a new intervention for brackishwater aquaculture development with immense potentiality. CIBA has also developed three tier farming model (nursery, pre-grow out and grow-out) and successfully demonstrated in different coastal states of India.

3.2.3 Polyculture of brackishwater fishes

KRC of ICAR-CIBA standardized polyfarming of brackishwater fishes with use of low cost farm made feed with locally available feed ingredients. This successful model of polyculture has been disseminated among the farmers of the Sundarbans, West Bengal, India paving the way for its wide adoption in the region. In this technology, six species polyculture with different stocking densities, *Liza parsia* (5000/ha), *Liza tade* (5000/ha), *Mugil cephalus* (2500/ha), *Scatophagus argus* (2500/ha), *Mystus gulio* (30000/ha) and *Penaeus monodon* (2500/ha), resulted 4764 kg/ha production using low cost farm made feed (Indian rupee, INR 25.32/ kg, USD-0.41/kg) having FCR of 1.36, in 325 days of culture period.

3.3 Diversified farming system

3.3.1 Integrated multi-trophic aquaculture (IMTA) / Brackishwater integrated farming systems (BIFS)

IMTA combines the farming of aquaculture species (e.g., finfish/shrimp) with organic extractive aquaculture species (e.g., shellfish/herbivorous fish) and inorganic extractive aquaculture species (e.g., seaweed / seagrass) in the appropriate proportions to create balanced systems for environmental sustainability, economic stability and social acceptability. The IMTA concept is very flexible and can be land-based (pond/RAS) or open-water systems (cage/pen), brackishwater or marine system. The terms 'IMTA' and 'integrated aquaculture' differ primarily in their degree of descriptiveness. The aim is to increase long term sustainability and profitability for the cultivation unit, as the waste of one crop is converted into fertilizer, food and energy for the other crops, which can in turn be sold in market. Understanding its potentiality and sustainable nature, all the stakeholders of coastal and marine aquaculture should be encouraged to promote it. Recently, ICAR-CIBA has conducted experiments and demonstration to popularize IMTA.

3.3.4. Biofloc-based farming system

The principle of biofloc technology is based on manipulation of carbon: nitrogen ratio (C: N ratio) and for brackishwater shrimp aquaculture C: N ratio of 10: 1 is stated to be optimum. The biofloc is heterogenous mixture of bacteria, algae, protozoa, zooplankton, food particles and dead cells with bacteria being the dominated component. The cultured shrimp often use the floc particles as their feed. For management of C:N ratio, carbohydrate is applied externally by different source including molasses, rice flour, wheat flour, tapioca powder, rice bran, wheat bran, etc. In presence of higher carbohydrate, the heterotrophic bacteria utilize ammonia to produce biofloc and thus reducing the level of free ammonia in the water. So, the chances of ammonia toxicity are reduced. This culture system improves the growth rate of cultured shrimp and fish. Apart from these, biofloc based system reduces the feed requirement leading to reduction of input cost and it also lowers the possibility of diseases. Research work carried out at CIBA showed that biofloc improved the growth rate of juvenile and adult *Penaeus monodon* by 29 and 12.6%, respectively over the control. However, this type of production system produces high level of turbidity, which increases the need of aeration. The dissolved oxygen (DO) level should strictly be monitored regularly and the aeration should be done round the clock (24 hours a day) particularly at the end of the culture period.

3.3.5 Periphyton supported farming

Periphyton based farming is widely used in fresh water aquaculture, particularly in carps, tilapia and giant fresh water prawn to augment fish production. Similarly, promising result in terms of growth, survival and production was observed with periphyton in brackishwater penaeid shrimp, *Penaeus monodon* (Khatoon et al, 2009) and *Litopenaeus vannamei* (Audelo-Naranjo et al. 2011). Like biofloc, periphyton is also a heterogenous mixture of biota including bacteria, fungi, phytoplankton, zooplankton, benthic organisms, detritus, etc. But unlike biofloc-based system, here the mixture of biota is generally attached to any submerged surface such as bamboo stick, plastic sheet, polyvinyl chloride (PVC) pipe, ceramic tile, fibrous scrubber, etc. Periphyton-based system also increases the aquaculture production and develops the resistances to different diseases by augmentation of immune response.

3.4 Brackishwater ornamental fish culture

Brackishwater ornamental fishes, like spotted scat (*Scatophagus argus*), moony fish (*Monodactylus argenteus*), crescent perch (*Terapon jarbua*), green chromide (*Etroplus suratensis*), orange chromide, *E. maculatus*, Puffer fish (*Tetradon cutcutia*), Knight goby (*Stigmatogobius sadanundio*), Four-banded tigerfish (*Datnioides polota*), and Green pufferfish (*Dichotomyctere fluviatilis*) are commercially important. CIBA has developed the captive seed production technology of scat, moony fish, crescent perch, green chromid and orange chromid.

A new avenue has been made by the successful breeding and seed production of spotted scat, perch and pearl spot by ICAR-CIBA.

3.5 Way forward

Further steps to develop sustainable brackishwater aquaculture are expansion of brackishwater aquaculture in inland saline areas, bringing more areas under culture, species diversification. Adequate availability of quality fish seeds will also help in expansion of culture. Development of eco-friendly and cost-effective culture technologies targeting small-scale farmers is the need of the hour. Development of intensive farming technologies like re-circulatory aquaculture system (RAS), improved polyculture, biofloc based farming, IMTA, organic farming, periphyton based culture will help to develop sustainable and eco-friendly brackishwater aquaculture.

2

An Overview of induced breeding techniques of Brackishwater finfishes

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Introduction

Successful aquaculture largely depends on the availability of sufficient quality seed at the required time. Availability of quality seed from natural sources is always erratic and undependable. Moreover collection of wild seed will deplete the natural fishery. Almost all of the cultivable brackishwater finfishes do not breed in captivity even though they attain gonadal maturity. Hence it has become necessary to go for induced breeding either by reproductive hormonal or environmental manipulation. Artificial spawning was first achieved in Italy during 1930 in striped mullet. Use of hormones to induce fish to spawn was started in Brazil in 1932. Compared to the advancement made in the breeding and seed production of freshwater fishes, the technology development in brackishwater fishes especially in India is far behind and this is to some extent are due to the non-availability of facilities for the development of captive broodstock and lack of expertise.

Selection of breeders

Breeders can be obtained either from wild or from broodstock developed in captivity. One of the problems faced in induced breeding is that variations occur in the gonadal development among individual fish both in the wild and in broodstock developed in captivity. Successful induced breeding depends upon the selection of the recipient fish at the proper stage of the gonad development. Normally, the external characters like fullness of belly, colour and state of swelling of genital opening such as protruding pinkish/reddish, genital papilla, softness and resilence of the belly (in females), roughness of pectoral fins, presence of hard tubercles (in males) etc. were considered for the selection of breeders. However, many of these parameters are not absolutely reliable. For example, enlargement of belly can be due to presence of food in the intestine and stomach. The more reliable method to assess the maturity of females now being used is through ovarian biopsy taking a sample of the ova

using a catheter and to examine them under microscope. The mature ova will have round shape and non-adhesive. The average ova diameter has to be determined and this is used as an important criterion in the selection of females for induced spawning. In the case of males, maturity is ascertained by applying pressure on either side of the belly. In the case of fish in mature condition, milt will be flowing through the genital opening on application of gentle pressure.

Sex determination

Majority of sea bass in the size range of 1.5 to 3.0 kg are males and as they attain a size of 3.5 to 4.0 kg, majority of them undergo sex change and become females. So, the size of the fish is commonly used for the identification of the sexes. Otherwise sexual dimorphism is not well marked and sex can be determined accurately only when they are in mature stage. In mature males, milt will be extruding on application of pressure on the abdomen. Females can be identified from the comparatively big soft round belly with pinkish genital papilla. In fully mature female, eggs will be even visible when the abdomen is pressed. There are some other minor identification marks. In males the snout is slightly curved while that of the female is straight. The scales near the cloaca of males are thicker than the scales in females during the spawning season. The body of males is comparatively slender compared to females. In the case of other fishes like Cobia and mullet, females appear with bulged soft belly with genital papilla. Males will be oozing while pressing the abdomen in both the species and also in milkfish.

Methods of Breeding

There are three methods by which fertilized eggs are obtained and seed production is done. They are artificial fertilization by striping of mature females and males, induced breeding by reproductive hormone administration and breeding by environmental manipulation.

Artificial fertilization by striping

In this method spawners are obtained from wild during the natural breeding season. In seabass breeding is related to lunar cycle. Again breeding occurs before midnight during high tide. Even though the fish breeds both during the new moon and full moon phases, quality of eggs released during full moon phase is better and the number of eggs released also will be more. Fishes caught during full moon and new moon phases and during high tide are examined for maturity. Both males and females that are in oozing stage can be striped and

fertilized artificially. In oozing females the diameter of the eggs will be around 0.7 to 0.8 mm with large oil globule. The eggs will be almost transparent. The ripe eggs will scatter individually whereas unripe eggs tend to group together in water. In water having salinity 28-30 ppt the ripe eggs will float.

For easy handling the selected females and males are anaesthetized. Eggs and milt are stripped into a dry clean tray and mixed thoroughly with a feather. After 1 – 2 minutes, fresh clean seawater of salinity around 30 ppt is added to keep all eggs floating and mixed well for 2-3 minutes. Then the eggs are washed 3 to 4 times using a strain to remove all mucus and other tissues. Thereafter the fertilized eggs are distributed to incubation tanks.

Environmental manipulation

This technique is usually followed in broodstock developed in captivity. About a month prior to the spawning season, the mature females and males are transformed to spawning tanks at a density of 1 kg/m^3. The salinity of the broodstock tank and spawning tank should be same. After 2 to 3 days when the fish got acclimatized to the spawning tank conditions, the salinity of the water is reduced to around 24 ppt. The fishes are maintained in this condition for about a week and then the salinity is gradually increased to 30 to 32 ppt by daily water exchange over a period of 10 days. This increasing of salinity simulates the condition similar to that of the migration of the fish from low saline feeding ground in the brackishwater to the high saline spawning ground in the sea and stimulates breeding.

On the ensuing full moon/new moon day, the water level is reduced to about 30 cm during noon time and the water temperature is allowed to go up to above 30^0C. By dusk fresh sea water is added to the spawning tank to simulate the rising tide conditions and simultaneously water temperature also declines to around 27^0C. The fish that is in right stage and good condition will spawn in the same night or during the subsequent night. The fish would continue to spawn for 3-5 days after the first spawning provided the environmental factors remain conducive. Seabass being an intermittent spawner releases eggs in batches; the same spawner will continue to spawn during full moon or new moon for the next 4-5 months. The fish that have not spawned can be subjected to induced spawning by hormone administration.

Induced Spawning

Seabass does not spawn in the broodstock tanks normally. Administration of reproductive hormones becomes necessary for inducing them to spawn. Human chorionic

Gonadotropin (HCG), Puberogen, Pregnyl and Luteinizing hormone – releasing hormone analogue (LHRH-a) are the main reliable synthetic hormones that are used for induced breeding.

The fishes that have to be induced are transferred from broodstock tanks to pre-spawning tank 2 months before the breeding season. These fishes are checked at fortnightly intervals to assess the maturity condition. The maturities of females are examined by taking out a sample of the eggs using a polyethylene cannula of 1.2 mm diameter. To avoid any handling stress, the fish is anaesthetized before the eggs sample is taken. Otherwise the head of the fish is inserted in a loose perforated plastic hood. The hood will extend upto the middle of the body. The fish is kept upside down keeping the head in water and the cannula is inserted into the oviduct. Since seabass releases 3-4 batches of eggs during the spawning process at definite intervals, it is clear that all the eggs in the ovary will not be in the same stage of maturity. Since the eggs in the posterior end of the ovary will get released first they will be in a more advanced stage of maturity compared to the eggs in the anterior region. Hence it is essential that the eggs in the posterior end are sampled while examining the maturity condition by inserting the cannula for a distance of 3-4 cm from the cloaca. The other end of the cannula is held in the mouth of the operator and the eggs are aspirated into the tube by the operator. When the eggs enters the cannula, the cannula is slowly withdrawn and empty the eggs slowly by the operator to a clear petri dish containing clean seawater and the diameter of the eggs are measured under a microscope using an ocular micrometer. Mature eggs get scattered around once it is transferred to a petri dish having water. Females that are having eggs of 0.4-0.5 mm average diameter can be given hormone treatment for induced breeding. Males with oozing milt are taken for breeding.

At Central Institute of Brackishwater Aquaculture, Chennai, des – Gly 10 (D-Ala 6) luteinizing hormone releasing hormone ethylamide acetate salt (LHRH-A) hormone is used for the induced breeding of seabass. Breeding is normally taken up on new moon or full moon nights. Female and male breeders are selected in the ratio 1:2 in the broodstock tanks and transferred to the hatchery. Their total length and weight are recorded and also ascertained that they are in good health condition. LHRH-A is administered to females and males @ 60 – 70 ug/kg body weight and 30 – 40 ug/kg body weight respectively and transferred to the spawning tank. Water salinity 30 – 32 ppt was found to be optimum for spawning. The breeders should be free from disturbances like excess noise and human

movements. They spawn after 30 – 36 hrs of hormone administration. The spawning may continue for a week releasing 3 – 4 batches of eggs.

In the case of grey mullet *Mugil cephalus*, the first maturity can be observed in 2-3 years old fish. In natural condition, mullet maturation and spawning noticed during October to January in the east coast of India and during June-July in the west coast. Longer darker period and low temperature directly linked with the maturation of *M. cephalus*. Females with initial oocyte diameter of 600 µm and oozing males can be selected for induction of spawning through hormonal manipulation. Carp Pituitary extracts and LHRHa @ 20mg/kg and 200µg/kg body weight are used as priming and resolving doses for spawning. After ovulation, stripping of ovulated eggs is common practice followed. The stripped eggs are fertilized by mixing with milt obtained from males using bird feather by dry method. The floating fertilized eggs can be stocked in the incubation tanks for hatching. The newly hatched mullet larvae can be stocked in the larval rearing tanks to grow them to fry size in the hatchery

Milkfish mature in seawater at the age of 3 years. However, broodfishes with age of 5 plus years are usually selected for breeding purposes. Milkfish require higher temperature and longer day period for maturation, which is usually coincide with summer period. Milkfish can be bred through LHRHa hormone treatment @ 50 µg/kg body weight either with pellet implantation and intramuscular injection. The hormone treated milkfish spawn spontaneously in the tanks and fertilized eggs are pelagic and float in the water. The fertilized eggs hatch out between 22-24 hours of incubation period and the newly hatched larvae can be stocked in the larval rearing tanks for fry rearing.

Cobia is one of the most preferred marine fishes in the cages because of its rapid growth rate. The fish can grow 4-6 kg in one year under ideal condition in the cages. It can be cultured in deeper ponds with good water exchange. Cobia tolerates the salinity range from 15 to 35 ppt. It is widely farmed in Vietnam, Mexico, USA, Taiwan, China and other South East Asian countries. Cobia matures after attaining the age of 3 years. Sexes are separate. The females, which are having the initial oozyte diameter of 700 µm are considered ready for hormone induction. By applying hormone treatment with HCG @ 250-500 IU/kg body weight, cobia can be induced to spawn. Cobia larvae reach to three inch size fingerlings in 45 days period rearing in the hatchery and these fingerlings can be stocked in the cages or ponds for grow out culture.

3

Reproductive biology and endocrine control of reproduction in brackishwater fishes

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Introduction

Similar to other vertebrates, the reproductive cycle of fish is divided into two major stages viz. growth and maturation phases. The first stage includes growth phase and second stage includes maturation and release of oocytes and spermatozoa. These two stage are regulated by the series of hormones from brain-pituitary-gonad (BPG) axis. The secretion of gonadotropins (FSH and LH) from pituitary gland is controlled by the GnRHs of brain (Peter and Yu, 1997). GnRHs are the main neuropeptides regulating reproduction; act as integrators of external information from environment (temperature, water fall and social interactions). Dopamine (DA) has an inhibitory effect on release of the pituitary gonadotrophs (Chang and Jobin, 1994). The FSH and LH are released from pituitary, which acts on the gonad and stimulate the synthesis of sex steroids (androgen, estrogen and progestogen). These steroids ultimately influence the gonadal development.

Ovarian development

Three types of ovarian development namely synchronous, group-synchronous and asynchronous are reported in the fish (Wallace and Selman, 1981). Oocyte growth/development and maturation are two essential steps to complete, final maturation.

Oocyte growth/development:

Major stages during egg development/growth involved formation of primordial germ-cells (PGCs), oogonia cells, primary oocytes and secondary oocytes (Patino and Sullivan, 2002).

Oocyte maturation

After vitellogenesis, meiosis is resumed and proceeds till metaphase II. This process is called meiotic maturation or oocyte maturation. LH regulate the final maturation of gametes, through the production of MIH or MIS, (Nagahama, 1994).

Testicular development

Spermatogenesis and spermiation, is regulated by FSH, LH, sex steroid hormones, and other growth factors. FSH triggers Sertoli cell proliferation and differentiation, and the synthesis of insulin-like growth factor I, IGF-I or activin B, which act as autocrine and paracrine factors (Schulz and Miura, 2002). FHS and LH control the synthesis of 11 keto testosterone (11-KT) and MIS from the Leydig cells of the testes, respectively. 11-KT regulates spermatogenesis while MIS regulates sperm capaciation and spermiation (Miura and Miura, 2003).

Brain pituitary gonad axis (BPG-axis)

Pituitary gland activity is regulated by neurohormones (neuropeptides, neurotransmitters), which is synthesized in hypothalamus of brain. In fish neurosecretory fibers connect brain and pituitary. These neurosecretory fibers are axon from the neuron of hypothalamus.

Pituitary gland

The pituitary gland orhypophysis of teleost is located in bony cavity, posterior to the optic chisma (Frisen, 1967). The pituitary gland, or hypophysis, comprises of the adenohypophysis and the neurohypophysis. The adenohypophysis is divided into three main parts: the rostral pars distalis, (RPD), proximal paras distalis (PPD) and paras intermedia (PI). In tetrapods, hypothalamo-pituitary portal system is present whose primary plexus of capillariesis located in the median eminence. The median eminenceis located in the floor of the hypothalamus. The neurohormones secreted from the hypophysiotropic neurons reached the target cells of anterior lobe of pituitary gland via blood. Posterior lobe of tetrapod pituitary is comprised of nerve fibers originated from hypothalamus, which secrete oxytocin and vasopressin. This part of the pituitary is commonly called as neurointermediate lobe. Major pituitary hormones associated to reproduction (LH, FSH) directly regulate the gonadal development in vertebrates. Secondary hormones associated to reproduction (GH or TSH) directly regulate other physiological process, and indirectly influence reproduction.

Reproductive hormones

Gonadotropin releasing hormone

The team of Nobel laureates Guillemin and Schally in 1970 characterized hypothalamic hypophysiotropic decapaeited peptides and named it luteinizing hormone

releasing hormone (LHRH), which stimulate the secretion of LH from pituitary gonadotrophic cells. Later it is found that LHRH also regulates other gonadotropin, FSH. This changes the name of LHRH to GnRH (Guillemin, 2005). Relative observation in different vertebrates showed the existence of different molecular variants of GnRH deca-peptides, with similar function to stimulate the release of pituitary gonadotropin (Millar, 2005; Kah *et al.*, 2007). Among vertebrates, teleost having maximum number of GnRH isoforms. Based on phylogenetic analysis three form of GnRH is found in vertebrates. Expression of GnRH-1 is seen in the hypothalamus of amphibians, mammals and fishes. Expression of GnRH-2 found in synencephalon/mesencephalon of all vertebrates from fish to mammals. Expression of GnRH-3 found mainly in the rostral forebrain of fish (salmon). Genes of GnRH have a common structure with 4 exons and 3 introns. Coding region of the GnRH genes is highly conserved but intron, upstream and downstream regions are distinctively divergent (Chow *et al.,* 1998).

Fish gonadotropin (GtH-I/ FSH and GtH-II/LH)

Gonadotropin are heterodimeric glycoproteins formed from two subunit of 'a' and 'b'. Subunit 'a' is common in both FSH and LH, which is non-covalently linked to b- subunit. The common a-subunit of both FSH and LH, b-sub unit of FSH and b-subunit of LH is encoded by a distinct gene. The a-subunit is the most conserved among fish at the amino acid level (Li and Ford, 1998). In fish, a-subunit has two potential sites for N-glycosylation and ten conserved cysteins, which form five inta-molecular disulphide bridges that are similar to mammals. During vitellogenesis, FSH induces steroidogenesis in two-cell model (outer theca and inner granulosa cells). The FSH stimulates the incorporation of Vtg into oocyte follicles (Jalabert, 2005). At the end of vitellogenesis, LH acts on oocyte follicle and trigger the synthesis and secretion MIH or MIS that regulate ovulation in female (Nagahama *et al.,* 1994; Suwa and Yamashita, 2007).

Hormonal changes during the reproductive cycle

Gonadotropin is an important hormone, which regulates the gonad maturation therefore, the estimation of this hormone in pituitary and blood plasma is essential to investigate the reproductive physiology of fish. In fish, both FHS and LH are secreted differently during breeding / reproductive cycle.

FSH and LH in female

FSH is released during entire vitellogenesis, while LH remains low during vitellogenesis and attain peak before ovulation (Davies et al., 1995; Prat et al., 1996).

FSH and LH in male

Both, gonadotropins (FSH, LH) are equipotent to stimulate the synthesis of the androgens, 11- KT and testosterone (T) in males (Planas and Swanson, 1995). In males, levels of FSH are high during early spermatogenesis, attain maximum value during testicular growth phase and decline after spawning. Alternatively, LH is low at early spermatogenesis, increases during Spermiation, and attains peaks at the spawning season (Mylonas *et al.,* 1997).

Sex steroids

Reproductive hormones play vital roles in many reproductive physiological processes of vertebrates. In teleost, most common sex steroid hormones produced in gonadal tissue are E2, 11-KT and 17α, 20β- dihydroxy-4-pregnen-3-one (DHP). These hormones are produced under the control of pituitary gonadotropins, which are important for gametogenesis (Wallace and Browder, 1985; Agahama and Yamashita, 2008 ; Miura *et al.,* 1991).

Estradiol

E2 level remain high during late-vitellogenic and vitellogenic stage, and low during post-vitellogenic and hydrated stage in mullet (Kumar et al., 2015) A similar trend of E2 is noticed in other fish species (Pham *et al.,* 2012).

Testosterone

In female of many teleost elevated T is observed during the per-ovulatory period (Barry *et al.,* 1992). This pre-ovulatory rise in T is to increase the pre-spawning GTH surge (Kobayashi *et al.,* 1989). A decreasing trend of T prior to spawning is probably to shift in the steroidogenic pathway from C19 to C21 steroid synthesis, which coincide with spawning (Barry *et al.,* 1992).

In male fish, annual reproductive cycle have two peak of serum T. Pre-spawning peak of T is to stimulate secondary sexual behaviours, increase pituitary GTH levels, and serve as a precursor of 11-KT. The second rise of T found just prior to the spawning season, which coincide with increase in 11-KT level.

Ketotestosterone

In general it is assumed that 11-KT is a male specific hormone, however it is also found in female fishes Pacific salmon (Schmidt and Idler, 1962) and rainbow trout (Scott *et al.,* 1980). Level of 11-KT is at peak when testis is full of spermatozoa. 11-KT also helps in maintaining viability of the spermatozoa.

Maturation-inducing steroid

Progesterone is the precursor of C21 steroid (Scott *et al.*, 1983). This includes 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-DP), 17α, 20β, 21-trihydroxy-4-pregnen-3-one (20β-S), 20β dihydroprogesterone and 11-deoxycorticosterone (DOC). These are maturation-inducing steroid (MIS), which induces GVBD and FOM (Nagahama and Yamashita, 2008).

Conclusion and future direction

The knowledge on endocrine control of reproduction is required to understand the maturation pattern of captive reared stock. Further it helps in hormonal intervention for induced maturation and spawning of fish. Hormone level in fish is used as biomarker to understand the level of pollution in natural water bodies. Overall information on hormonal cycle will be helpful for captive breeding of fishes.

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4

Seed production and low cost hatchery technology of *Mystus gulio*

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Introduction

Brackishwater catfish locally known as "nuna tengra", (*Mystus gulio*) is an important small indigenous fish species (SIS) of the Sundarban delta. They inhibit in shoals in low saline water of estuarine and coastal areas. In natural water bodies, they feed on organic matters and small crustaceans. The best salinity for its farming is 5-12 ppt, where it attains a maximum size of 30 cm (250 g) in a year; however, they can thrive also in 1-2 ppt of salinity. This is an important candidate species for aquaculture diversification because of its hardy nature, delicious taste, excellent nutritional value and high market demand. To meet up this high demand and also to conserve this species, it is essential to develop production system under controlled condition. In this context, Kakdwip Research Centre of ICAR-Central Institute of Brackishwater Aquaculture has developed a comprehensive technology comprising of captive breeding, larval rearing and grow-out culture of this fish in brackishwater system.

Broodstock development

In captivity fish attain sexual maturity at the age of 6-8 months. However, the small size brooder (50-70 g) and will be low fecund (5000-8000). To get potential sizable brooder fish has to reared for a year, in which it will attain the size of more than 100 g. It is also advisable to collect wild adult (>50 g) during month of February to March that is before the onset of peak spawning season (May-August) and reared in small earthen pond (1000 m^2) at the density of 2/m^2. During broodstock development fish should be fed with high protein

pellet diet (30 % protein, 8% lipid) and/or liver @3% of biomass. Physico-chemical parameters of the pond water, such as salinity, temperature, pH, alkalinity and dissolved oxygen should be 5-20 ppt, 29 ± 1^0C, 7.5-8.0; 144 -150 ppm, 5-8 ppm, respectively.

Captive breeding

Selection of mature broodstock

Matured male and matured female fish in a sex ratio of 2:1 is selected for the induced breeding. Maturities of the females are staged by obtaining *in vivo* biopsy of ovary using a polyethylene cannula (2 mm diameter). Female having oocytes with average diameter of more than 900 µm are selected for induced breeding trial. Other ways to assess the maturity of female is through swollen belly, reddish open vent and round bulge anal opening. Mature male having elongated papillae with pink or red tip are selected for induced breeding.

Sex identification

Male and female sex was distinguished based on the presence of pointed genital papilla (Fig 1) and soft swollen abdomen with round vent (Fig 2), respectively.

Fig. 1. Male

Fig. 2. Female

Prophylactic treatments

Selected breeders are treated with a 50-ppm formalin bath for 3 min to remove unidentified external parasites and then transferred to breeding tank fitted with water circulatory system.

Hormone injection

After selection, males and females in the sex ratio of 2:1 are acclimatised in breeding tank water for 2-3 h. In breeding tank, round the clock aeration and water flow are maintained. For induction of spawning any of the inducing agents such as human chorionic

gonadotropin (HCG), LHRHa, pituitary gland extracts (PGE) or commercial inducing agents may be used. Dose of HCG, LHRHa and PGE are 10,000 IU, 35 µg and 10 mg per Kg of female. Half the dose of female is administered to male. Hormone dose depends on maturity stages of *M. gulio*. Hormone is injected into the dorsal musculature of fish adjacent to the first dorsal spine (Fig 3). Injection is generally given after noon.

Fig. 3. Hormonal injection

Fig. 4. Egg collector, made up of nylon fibres

Spawning and egg incubation

M. gulio spawned after the latency period of 10-12 h. The latency period is the time gap between hormone injection and the first appearance of spawned eggs. *M. gulio* fertilized eggs covered by a gelatinous material that gives adhesive properties to eggs. Fertilized eggs are transparent, adhesive, demersal, and spherical in shape, whereas unfertilized eggs are white in colour. The *M. gulio* egg yolk is devoid of structural lipids visible as droplets / oil globules. After spawning, flow through is switched off, and the egg collector (Fig 4) containing the sticky eggs are transferred to incubation tank. It is a low fecund fish and total fecundity ranges from 25000 to 150000 eggs, which depends on the size of the female.

Incubation tanks are arranged well in advance. After 3-4 h of spawning egg collectors are collected and transferred to incubation tanks (1000 L) fitted with aeration. Eggs started hatching after an incubation period of 17:30 h, at ambient temperature of 28 ^{0}C. Incubation period is temperature dependant, which may vary from 14-18 h. Newly hatched larvae measured 2.17 ± 0.29 mm in total length, with large yolk sac without oil globule. The newly hatched larvae were inactive and remained attached to the side and bottom of tank. After 6-12 of hatching, egg collectors are removed.

Larval rearing

To avoid the handling stress and mortality, hatching tanks are used as larval rearing tank. Egg collectors are removed 6-12 h after hatching. After 24 h post hatching (hph), larval rearing tanks are inoculated with *Chlorella spp.* at a density of 1-5×10^{3} cells ml^{-1}. After two days post-hatching (dph), larvae were fed with freshly hatched *Artemia spp.* nauplii measuring 150- 175 µm in width and 500-580 µm in total length. Optimum larval stocking density is 25 nos/ L. Larvae are fed with *Artemia* nauplii at the density of 3000 nos/ L for four times in a day for initial 7 days. *Artemia* nauplii and crumbled feed from 8 dph and exclusively with crumbled feed from 15 dph. In 30-35 dph, fry attain 48-50 mm size and cost of production for one fry varied from 30-40 Paise only.

Economic viability

Estimated total cost of production for one lakh seed is Rs. 50,000/-, with gross return of Rs. 60,000/- and net return of Rs. 10,000. Benefit Cost Ration (BCR) for one lakh seed production remain 1.2. Therefore in short period of 3 months total cost of production will be returned with net benefit of Rs. 10,000.

5

Breeding and seed production of brackishwater ornamental fishes of Sunderban

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1. Introduction

Ornamental fish industry is multibillion dollar industry with an export value of US$347.5 million during 2014. Asian countries forms 57% share of this trade with estimated value of US$197.7 million in exports. Ornamental fish industry plays an important role in terms of poverty alleviation and secondary income generation for small-scale aquafarmers and women self-help groups in developing countries. Singapore supplies 20% (US$69.32 million) of the ornamental fishes while USA imports 14.3% (US$42.9 million) fishes of total trade. India is blessed with a rich natural biodiversity of fish species including over 400 ornamental fish species however India's share in the ornamental fish trade is >0.1% of the global trade. More than 90% of the fishes in ornamental trade are freshwater habitants which are bred in captivity. Brackishwater fishes in ornamental fish trade are not widely popular. Due to euryhaline nature of these fishes, often they are sold for freshwater or marine aquaria. Natural habitats of brackishwater fishes constantly change its salinity, temperature and other water quality parameters. Therefore, these fishes naturally respond well to environmental stressors and this attribute can be harnessed for maintaining them in different salinities in aquaria.

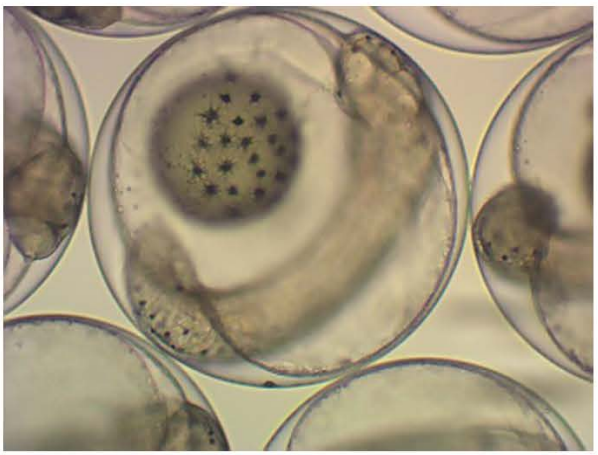
ICAR- CIBA has initiated R and D efforts to identify the indigenous brackishwater ornamental fishes and develop their juvenile production technology in captivity to create a niche. Presently, induced breeding and seed production technology of six indigenous ornamental brackishwater fish species; spotted scat (*Scatophagus argus*), silver moony (*Monodactylus argenteus*), orange chromide (*Pseudetroplus maculatus*), crescent perch (*Terapon jarbua*), Canara pearlspot (*Etroplus canarensis*), green chromide (*Etroplus suratensis*) have been standardized by ICAR-CIBA in captivity.

2. Captive breeding of indigenous brackishwater ornamental species at ICAR-CIBA

2.1. Spotted Scat (*Scatophagus argus*)

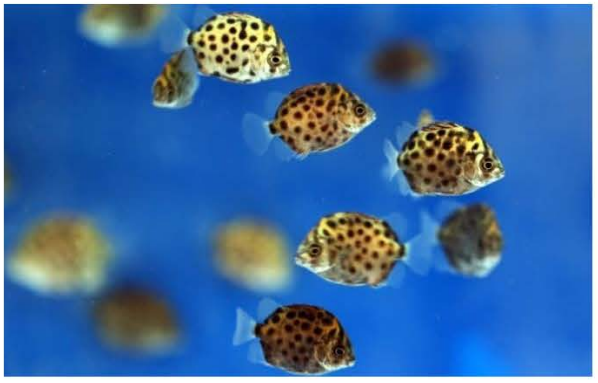
Spotted scat is distributed along Indo-pacific coastal waters and occurred commonly in mangrove and backwaters regions. It is also known as Green scat, Argus fish, Spade fish, Butter fish, and Tiger scat etc. popularly. It belongs to the order Perciformes and family Scatophagidae. It is a deep and compressed body fish with greenish – brown / silvery - golden on the sides with brown to reddish-brown spots which makes it an attractive ornamental fish. Two colour morphs; green and red has been reported by researchers. The fish adapts well with other fishes in aquaria. It is an herbivore fish in nature and readily accepts artificial feeds in captivity. It is also a candidate fish to be cultured with selected finfishes / shall fishes for food purposes. It possesses paired poison glands associated with each fin spine which can inflict pain for several hours on careless handling. Demands for juveniles in domestic and international market are high. Juveniles can be sold for ₹ 15 – 25/pc. Adult fishes >150g fetch premium price as food fishes. Its flesh is soft and tastes good. In aquaria adults and juveniles can be kept in varying salinities due to their euryhaline nature.

Brood Spotted Scat



Fertilized egg

Hatched out scat larvae



Hatchery produced Spotted scat

ICAR - CIBA has successfully developed protocols for captive maturation and induced breeding of spotted scat for larval production in captivity. In nature, first maturity is attained by +1 year age group fishes. Sexes can be differentiated morphologically in spawning season by bulged appearance of female and running milt from males. Adult fishes are spawning in coastal waters after onset of first monsoon in nature during April to October. The females are larger and heavier than the male during spawning season. Brood fishes selected for induced breeding range between 150 - 450 g in body weight. Adequate feeding and health monitoring is required for management of broodstock in ponds/tanks to accelerate the gonadal maturation. In the breeding season, females with oocyte size >450 – 550 µm are selected for induced spawning (Mandal et al., 2019). Pairs are maintained in 1 female: 2 Male in spawning tank. Females are administered with HCG twice @ 2000 IU/kg body weight with an interval of 24 hours as priming dose. LHRHa @ 400 µg/kg body weight induces the female for spawning. Male fishes are administered with LHRHa @ 200 µg/kg body weight (Mandal et al., 2021). Spawning is observed after 30 – 32 hours of last injection. Upto 80,000 - 1 lakh fertilized eggs (size: 700 – 750 µm) can be obtained from each female. Newly hatched larvae are about 1.6 mm in size which hatches post 18 – 24 hours incubation of fertilized eggs. Larvae are reared on rotifers from day 2 to 10, and afterwards with *Artemia* nauplii up to day 20 - 25. Fry are then weaned to formulated feed and a fry of marketable size is attained in 45 - 60 days.

2.3. Pearlspot (*Etroplus suratensis*)

Pearlspot, belonging to order Perciformes; family Cichlidae are one of the three indigenous cichlids of India. It is distributed in the Indian peninsular region and Sri Lanka. Pearlspot has grey-green colouration with white pearly spots all over the body. It is a popular food fish in Kerala and the juveniles are also considered as ornamental fish in domestic and international markets. It is also state fish of Kerala. Pearlspot exhibits parental care of the young ones and interesting behavior patterns as pairing and territorial defense. The seed production technology of the species in a modular tank based system has been standardized by CIBA. Breeding frequency of pearlspot is optimised through intervention like curtailing of parental care and specialized broodstock feeds. Production of upto 1000 fry / pair / month is observed from this system. Larval rearing is conducted using *Artemia* nauplii or starter feeds. Pearlspot larvae are being successfully rearing the by women and tribal groups as a subsidiary homestead activity for generating additional income with the technical guidance from CIBA.

Adult pearlspot

2.4. Orange chromide (*Pseuetroplus maculatus*)

The Orange chromide is an endemic species of freshwater and brackishwater streams, lagoons and estuaries in states of Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu in India. It belongs to order Perciformes and family Cichlidae. The fish is compressed, oval shaped and yellow to orange in colour. Large black pots are observed on the body. The fish exhibits parental care and the offsprings are taken care of by the parents. It attains a maximum total length of 80 mm. Fecundity of the fish is 1378 approximately and clutch size reported to be between 140 - 231 eggs/spawning. Size of the fishes varied from 6-10g in weight and 6-9 cm length. In nature the fish form a breeding pairs and attach eggs on the substrate.

Brood fish of Orange chromide

Deposited fertilized eggs

Fry of orange chromide

Presently, *E. maculatus* is being collected from the natural water bodies and sold in aquarium shops with price ranged from Rs.10 - 15/fish. Wild collection of *E. maculatus* is seasonal and inadequate in quantity. ICAR-CIBA has standardised a breeding model for a pair of orange chromide. For pairing and spawning, two pairs of orange chromide of male and female (2:1) were stocked in each tank. Spawning could be observed after 10-12 days of stocking in the broodstock tank. 250-300 numbers wriggler stage larvae (3 days post hatch) could be collected from each tank. 10 - 15 days after wrigglers collection, repeated spawning could be observed.

Average Fecundity is 292±109.38 and spawning frequency is 12 ± 2.13 days. The eggs are pale yellow and ellipsoidal in shape with an average size of 1.5 mm which attaches to the nest surface by a stalk. Incubation period is 46 - 48 hr. Newly hatched larvae are fed with rotifers up to 15 dph followed by *Artemia* nauplii up to 25 dph and later weaned to formulated diet. 45 – 60 dph larvae can be sold for trade purposes.

2.5. Canara pearlspot (*Etroplus canarensis)*

Etroplus canarensis is also known as Canara pearlspot / Banded chromide / Roman numeral cichlid is an important ornamental fish species listed as Endangered in the IUCN Red list Threatened species. It is endemic to South Karnataka in India and distributed in Kumaradhara and Nethravati rivers. In nature, sexual maturity reaches at 2 years and broodfishes are reported to breed during the December and January. Adult males are larger and heavier than female fishes.

Brood fishes of Canara pearlspot

Wrigglers of Canara pearlspot

Canara pearlspot was undertaken in ICAR – CIBA and the fish were successfully bred in captivity. In captivity, it laid eggs on a substrate and both parents cares for the filamentous stalked eggs attached to a substrate. Parents tirelessly fan the eggs with their pectoral fins and also clean the eggs by brushing them with fins. They remove any dead egg from the clutch. Fertilized eggs hatch after 4 days at temperature of about 28 - 30°C and 2 – 3 dph fry shows schooling behaviour. In captivity, 12 days old larvae were separated from parents and stocked @ 5 nos. /l in rearing tanks upto 60 days. *Artemia* nauplii were fed upto 30 dph and afterwards the fishes were weaned to formulated diet.

2.6. Crescent perch (*Terapon jarbua*)

The crescent perch belong to order Perciformes and family Teraponidae is distributed in the Indo-Pacific region. It is also called as Target Fish, Crescent Bass, Crescent Perch or Tiger Bass. It can be considered as brackishwater ornamental/food fish. The fish has three or four crescent shaped dark brown bands running from the nape to the hind part of the body which earned it the name 'Crescent perch'. Milting males and female with oocyte diameter exceeding 460 µm are used for induced breeding. Hormonal concentrations of HCG and LHRHa have been optimised for successful induced breeding. Female spawns semi-buoyant eggs (fertilized egg size: 750 µm) after 36 h post injection. Hatching of 2 mm hatchlings occurs after 16-18 h of incubation. Upto 3 lakhs larvae are obtained per spawning. Larvae are fed on rotifer *Brachionus plicatilis* and later weaned to artificial feeds.

Brood crescent perch

Hatched out larvae

6

Induce breeding, improved larval rearing and hatchery seed production of milkfish (*Chanos chanos*)

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Concepts

1. Milkfish (*Chanos chanos*) is an important candidate species suitable for brackishwater aquaculture. It is an herbivore fish, tolerate wide range of salinity and accept low protein pellet feed under culture conditions. During 2015, ICAR-CIBA achieved breakthrough in captive breeding of milkfish and established seed production technology.
2. Captive land based broodstock of milkfishes are maintained in 100t capacity RCC seawater tanks and periodically induced breed with application of combined hormone pellet consisted of GnRHa and 17α-Methyl Testosterone hormones (each 50 µg/kg). After combined hormone treatment, mature females (mean oocytes diameter 680-700 µm) and oozing males could be observed under captive conditions.
3. Milkfishes are batch spawner and perform community-based breeding. In captivity eight months spawning could be observed during February to September. Eggs are pelagic and incubated for 24-26 h to hatch out. Total length of the newly hatched larvae is 3.2-3.4 mm.
4. Milkfish larvae are reared in green water system, fed with rotifer and artemia till they reach to weaned fry (2-2.5 cm) stage in 3-4 weeks. Milkfish fry can be stocked in outdoor nursery ponds in different salinities and can be fed on benthic algae, periphytons, artificial feed etc. After 4-6 weeks of nursery rearing in pond milkfish fry attains 6 – 10 cm (fingerling) body size and can be subsequently transferred to grow out ponds/pens.
5. Milkfish can be monoculture or polyculture with other species and grow to marketable size of 500 g in six months period with productivity of 2 - 4 t/ha. Hatchery produced quality milkfish seed is available during off seasons in CIBA fish hatchery has driven interest of many farmers and entrepreneurs.

6. Hatchery produced seed distributed among farmers from Kerala, West Bengal, Gujarat, Andhra Pradesh, Goa, Maharashtra, Tamil Nadu and Karnataka for culture trials and demonstrations. In a milestone achievement, ICAR-CIBA partnership with Raj Hatchery, West Bengal for milkfish hatchery technology in east coast of India.

1. Introduction

Species diversification is the key element for sustainability in brackishwater aquaculture. Finfishes can be viable alternate to shrimp dominated brackishwater aquaculture sector. Finfish farming based on species feeds on lower trophic level such as milkfish, mullet have huge potentiality due to less production cost and ready market. Milkfish is herbivore in feeding nature, which feed at lower tropical level on benthic algae (*lab-lab*) and phytoplankton. Due to euryhaline nature this fish can tolerate a wide range of salinity. Milkfish is an important food fish traditionally cultured in Southeast Asian countries, particularly the Philippines, Taiwan and Indonesia. Global production of Milkfish was about 1188 thousand ton during 2018 (CIBA AQUASTAT- 2019) and forms 2% of global finfish production. Milkfish belongs to the order Gonorynchiformes and is the only live species of the family Chanidae. In nature, adult milkfish are found in marine systems with distinguished and prominent features like: i) Elongated, moderately compressed, smooth and streamlined body ii) An adipose layer on eyes iii) Attractive silvery body colour and sides grading to olive-green or blue iii) Deeply forked large caudal fin. In India, milkfish is minor fishery and the availability of wild seed is seasonal, scanty and often mixed with predatory species. Occurrence of fry and fingerling and its traditional culture has been reported in coastal waters, estuaries and brackishwater water bodies of peninsular India since many decades back (Tampi, 1957 and Silas et al., 1985). It is called as *Paal Meen* in Tamil, *Pala Bontha* and *Tulli Chepa* in Telugu, *Poomeen* in Malayalam, *Hoomeenu* in Kannada, *Golsi* in Goa and *Sebakhainga* in Oriya. Realizing the potential of milkfish farming in the underutilized brackishwater and inland saline waters of India, CIBA initiated broodstock development of milkfish since decade back and achieved breakthrough in captive breeding and seed production of this species in year 2015 (Bera et al., 2019a). Hatchery technology perfected in consecutive years and availability of milkfish seed has triggered the scientific farming in different coastal states of India. Milkfish accepts low protein pellet (25-27 % crude protein) feed under culture conditions and grows to marketable size of 400-500 g in a six-months of culture period. Due to similar morphological appearance and spiny nature like hilsa it promoted as *Decan Hilsa* in domestic market of West Bengal. It has a ready market with a

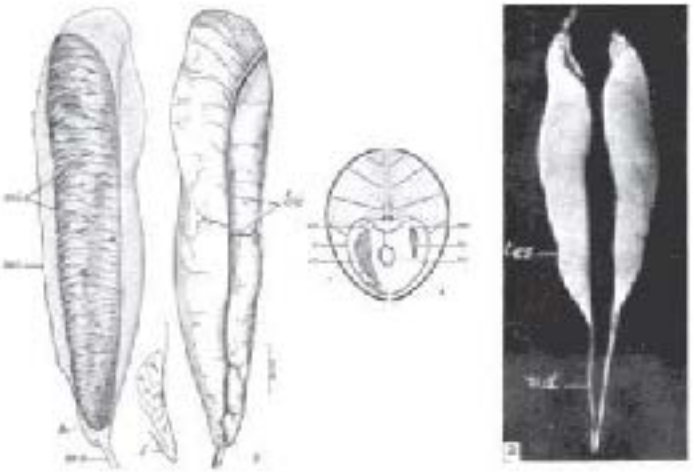
selling price of Rs.180-240/Kg in Kerala, West Bengal, Odisha and other North Eastern states of India.

2. Maturation, Induced breeding and larval rearing

Major constrains to achieve mass artificial propagation age generally are limited resource of mature broodstock, proper environmental manipulation and stress from frequent handling, brood nutrition management and administration of required dose of synthetic hormones are few. Natural maturation and spawning have taken place in floating sea cages and more notably, in earthen marine ponds in recent past. In a path breaking experimental trial Lee *et al.* (1986) tested five chronic hormone therapies and found that a combination of LHRH-a cholesterol pellets and 17α- Methyl Testosterone (17α-MT) capsules effectively stimulated maturation of milkfish and induced them to spawn under captivity. The lack of commercial scale availability of hatchery produced seed is the major bottleneck for any large-scale venture of marine finfish farming. The availability of seeds from wild is often unpredictable, risky as they come with predator fish, un-uniform size group and hence farming based on wild collected seeds may not be a sustainable venture. Hence the development and standardization of seed production techniques milkfish should receive research priority. (Lee *et al*., 1986; Tamau, 1988).

3. Sexual Dimorphism

Milkfish being a bisexual fish mature males have, 2 openings in the anal region which are externally visible in, and 3 in mature females. Female Milkfish attain sexual maturity at around 5 years of age whereas males maturity earlier at around 4 years of age.



Ovary and testis of mature milkfish

Source: The Proceedings of the Indian Academy of Science (Tampi, 1957)

4. Maturity Determination

Fishes fail to achieve their normal reproductive cycle under captivity. Culture conditions much time do not provide an environment conducive to completing maturation of the gonads and ultimately spawning. In some cases, changing the environment like maintaining of salinity, temperature, water quality parameters has proven sufficient alteration for fish to resume their normal reproductive activities. Milkfish do not exhibit any sexual dimorphism and thus adult milkfish of 6-8 years old should randomly stock into cement/FRP tanks with an assumed sex ratio of 2:1 (M:F). Sometime salinity affects ovulation and may exert some influence on gonadal development when it is extremely high. Temperature (240 C is minimum) and photoperiod (11L: 13D; 14L: 10D) is very important parameters but less studied. It has been seen that gonadal maturation is synchronised with temperature rising from 25 – 32^{o} C and 11–14-hour light.

Problems and solutions related to milkfish maturation

- Stress due to frequent handling seemed to be negative for the stimulatory effect of hormones and initiates resorption of gonads in both sexes.
- Pellet implantation and hormone medicated feeding exerts lesser stress. But Protein (Gonadotrophins : LHRH, GnRH, FSH) based hormones cannot be fed orally but sex-steroids can be administered in that way.
- It was seen that spermatogenesis can be obtained in 4-year old milkfish through feeding of 17 alpha-methyl testosterone.
- Milkfish <4 or 5 years old may not have developed receptors to respond to hormone treatments.
- In wild collection ripe males are less in numbers. If not hormone induced, males reabsorb milt after 2-3 days. To induce spermiation HCG + androgens can also be used.
- It has been seen, milkfish sperms can be stored for 10 days in 12.5 % DMSO at near zero temperature. As male fishes mature early than female cryopreservation of milt may be of great help. (Lam, 1984)

5. Brood Nutrition

The broodstock feed for milk fish, Milkfish Brood ^{Plus} has been formulated and developed by CIBA to support sexual maturation and production of quality eggs. The milkfish Brood ^{Plus} prepared using quality ingredients comprising marine and plant protein sources, energy sources and enriched with vitamin A, vitamin C, phospholipids and carotenoids. The diet is prepared as extruded floating pellets and it contains 38% protein with

8% lipid. Milkfish broodstock was fed with milkfish Brood ^{Plus} @ of 3% of the body weight in two equally divided doses at morning and evening.

6. Induction of Gonadal Maturation and Induced Breeding

Induced maturation of milkfish commonly starts at oocyte dia of 0.66 - 0.82 mm at 35 ppt salinity. Sex ratio of 2:1 (M:F) is better to maintain. Fertilized eggs are represented by 1.1-1.23 mm oocyte dia. Fish handling stress can be minimized by 2- phenoxy ethanol or diazepam as tranquilizers during handling. The hormones used can be Salmon or Carp pituitary homogenate in combination with HCG, or HCG alone. Hormone dosage can be variable: (Lam, 1984)(a) SPH 6-10 mg/kg,(b) CPH 5-25 mg/kg,(c) HCG 180-2500 IU/kg. Numbers of hormone injections are generally 1-5 (mostly 2) with injection interval of 6-24 h (mostly 8-12 h). Time to stripping is commonly 6-17 h (12 h appears best) in case of oozing female and male. There are certain behavioral markers which may help to determine both whether the injection given is effective and also the time of stripping. These include the following (Lam, 1984):

- Color change, due to melanophore stimulating hormone (MSH) in the pituitary homogenate
- Increased drinking activity, probably to facilitate oocyte hydration
- Release of calcium deposits, probably with increased drinking; calcium is retained in the gut and then released
- Distension of abdomen, indicating oocyte hydration
- Dribbling of some eggs, indicating that ovulation may be close, consequently
- A good reference point to determine the time of stripping.
- Milkfish less than 4 or 5 years old may not have developed the receptors to respond to hormone treatments.

Similarly, spent fish may lack hormone receptors. It is not known how long it takes spent fish to undergo recrudescence (rematuration) or whether they remature at all in captivity. It is also not certain whether milkfish are total or intermittent spawners. ICAR-CIBA has achieved induced breeding of Milkfish first time in India during June, 2015. Brood stocks of milkfishes were maintained in 100 t capacity cement tank for more than 8-10 years at Muttukadu Experimental Station of CIBA. After several number of LHRH-a hormone pellet implantations final gonadal maturity and first successful spawning of milkfish were happened. Fertilized egg diameter was 1.23 mm and length of newly hatched larvae was 3.4

mm. Successful maintenance of brood stock is one of the key factors in hatchery production of milkfish seed.

7. Induced breeding, spawning and hatching

7.1. Induced breeding

Natural spawning using pond-reared milkfish was achieved in Taiwan. The first instance was in August 1980, when the milkfish broodstock in floating cages at SEAFDEC/AQD spawned spontaneously. When mature, the ovary is usually around 10% of body weight, but could be nearly 25%. A 5-13 kg female can produce 300000 eggs/kg body weight. In wild, milkfish spawns more than once a year. Spontaneous spawning without hormone treatment has also been achieved with captive broodstock maintained in floating net-cages in the Philippines.

ICAR-CIBA achieved breakthrough in induced breeding of Milkfish

At fish hatchery, fertilized eggs of milkfish (*Chanos chanos*) generally obtained from hormone pellet implanted more than 10 years old broodstocks (Total 16 numbers, 4.4 – 7.2 kg) maintained in 144 t capacity open RCC tank at Muttukadu Experimental Station of ICAR- CIBA, Chennai. Eggs (1.24 mm dia) are collected from egg collection tank during early morning (6 am) and incubated in 500 l capacity conical FRP tanks with mild flow through (1.75 l/min) of filtered seawater (Salinity 32 ppt, Temperature 27° C - 29° C) and constant aeration to facilitate movement and floatation of eggs to hatch out within 25 h – 26 h after fertilization (Lee et al., 1986a & 1986b). ICAR-CIBA achieved captive breeding and seed production of milkfish *Chanos chanos* for the first time in India during 2015. Anesthetized fishes with 2-Phenoxyethanol (300 ppm) can be implanted intramuscularly with combined hormone pellet consists of LHRHa and 17α-Methyl Testosterone (17 α- MT) hormones (each 50 µg/kg). Weight of each combined hormone pellet is 23 mg containing cholesterol as matrix, hormone (LHRHa and 17α- MT) as inducing agent and coco butter as binder. Cylindrical hormone pellets are 5.0-5.1 mm long with 2.0-2.1 mm diameter and provides sustained release of hormone to accelerate maturation. Hormone pellets were prepared after modifying methods reported previously (Lee et al. 1986a, 1986b). Hormone pellet is implanted twice or thrice at 30 days interval during breeding season to attain the maturity. Mature females (mean oocytes diameter 680-700 µm) and oozing males could be observed under captive conditions after treatment with combined hormone and selected for breeding. Post implantation, betadine/cipladine and vaseline ointments can be applied at the

implanted region to avoid secondary infection and entry of water. Fish will be able to recover from aesthesia and exhibit normal swimming after few minutes (Bera et al., 2019b ; Bera et al., 2021).

7.2. Spawning

Milkfishes are multiple spawner and perform community based breeding in 100t RCC tanks. After 2-3 months of combined hormone treatment, mature females (mean oocytes diameter 680 µm) and oozing males is observed under captive conditions. In captivity, eight months spawning from February to September is possible. Circular egg collection chamber (500 lit capacity) is placed separately and fitted with plankton net to prevent escape of any eggs. Floating eggs can be collected in egg collection tank in early morning. Due to spawning inside open tanks eggs collected during broodstock tank draining can be make free of debris by settling them in separate chamber. In natural spawning of milkfish, fertilization is 70 - 90%. The size of the fertilized eggs is around 1.20 – 1.24 mm. The fertilized eggs are round, transparent and buoyant in nature. The unfertilized eggs are opaque and slowly sink to bottom. Due to water hardening and higher water salinity even, unfertilized eggs, for short duration remain on the sub-surface but sink subsequently. In an average 14-18 spawning could be observed during February to September with egg production of 1-1.5 lakhs eggs/spawning and total spawning of 1.5-2 million eggs in a season (Bera et al., 2021).

7.3. Incubation and hatching

The eggs collected from the spawning tank are washed to remove the debris and prevent its transfer to the incubation tanks. The incubation cum hatching tanks are cylindro-conical in shape with water holding capacity of 200 l. The eggs are stocked @ 500 nos/l. Continuous aeration and mild flow through (1.75 l/min) of filtered seawater is provided at 27°C - 29°C is desirable. Incubation period of milkfish is 24-26 hours after fertilization.

8. Improved larval rearing of milkfish

Newly hatched milkfish larvae having a large yolk sac with initial mean total length (TL) of 3.4 ± 0.06 mm were collected from incubation tank and stocked in RCC tanks at the rate of 2.5 larvae L^{-1}. Phytoplankton, *Chlorella salina* with mass culture cell density of 0.4-0.5 million cells ml^{-1} are introduced in LRTs from 2 dph to 20 dph to maintain the green water with a cell density of 10^3 to 10^4 cells ml^{-1}. Mass cultured *Brachionus plicatilis* (140 – 210 µm) are collected and enriched with frozen green algae paste *Nannochloropsis oculata* (Nanno 3600 ™, 68 billion cells/ml) (Thépot, 2016) and fresh *Chlorella salina* (1:1 ratio) in

a 100 L volume container overnight and are supplied as initial feed to the larvae @ 20-30 numbers ml^{-1} from 3 dph to 14 dph . Larvae can be co-fed with rotifers until 15 dph during the rotifer–Artemia transition (Woolley et al., 2012). Newly hatched *Artemia* nauplii@ 0.5–1.0 numbers ml^{-1} generally introduced from 15 dph till 20 dph following initiation of weaning to artificial feed (200-300 µm) from 21 dph. Water exchange initiates from 6 dph, initially at 10 %, and increased to 50 % by 20 dph. Rotifer and *Artemia* nauplii are added two times a day only (0700 h & 1600 h). Prey can be counted every 3 h till 20 dph and any reduction recorded in the prey density adjust by adding the required feed in the experimental tanks following the above feeding schedule. Sufficient aeration provides in LRTs to ensure homogenous distribution of algae and live feeds throughout the water column. Sand filtered seawater at 28° C - 29° C are used for entire rearing and important water quality parameters are always in optimum range.

9. Live transport of fertilized egg

Eggs (embryonic stage) and newly-hatched larvae were also transported in oxygenated plastic bags containing 12 L seawater and supported by straw bags. Optimum density, temperature, and salinity should maintain are: 100,000- 120,000 eggs or larvae per bag, 28-30°C and 32-34 ppt of salinity.

Way forward

Milkfish being herbivorous euryhaline species is slowly getting position as an important cultivable species tin brackish water sector in India. As nursery rearing, pre grow out and grow out culture of milkfish is easy, less risky, less capital intensive and good economical return, it can lead to a sustainable culture option in vast underutilized saline areas apart from shrimp farming areas in India. Successful induced breeding and commercial level seed production of milkfish can open a new horizon to include more species for brackishwater aquaculture. Milkfish can be cultured at coastal waters, estuaries and brackishwater water bodies such as Chlika lake in Odisha, Pulicat lake in Andhra Pradesh, Bheries in West Bengal, backwater in Kerala, Goa and Karnataka. Milkfish having similar look and spiny nature as *Hilsa* can have a ready market with a selling price of Rs.150-200/Kg in West Bengal, Odisha and other North Eastern states of India and can be recognized as *Decan Hilsa* in domestic market. Milkfish can be used as live bait for tuna industry also.

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7

Present status of milkfish (*Chanos chanos*) farming

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1. Introduction

The milkfish (*Chanos chanos*) is one of the most ideal finfishes for farming in coastal areas. They are fast growing, tolerates a wide range of temperature, oxygen and salinity. Milkfish are cultured in large scales in countries like Indonesia, Philippines and Taiwan in ponds called "Tambak". Milkfish farming in Indonesia, Taiwan Province of China and the Philippines started about 4-6 centuries ago. In India to the popularity of its farming is growing especially in Tamil Nadu and Kerala. The fish is either monoculture or polyculture with compatible species of fish & shrimps.

Nursery operations in milkfish producing countries vary according to established cultural practices. In Taiwan Province of China, where commercial hatchery and nursery productions are integrated enterprises, milkfish fry are generally grown in either earthen ponds or elevated canvas or concrete tanks at intensive stocking densities of >2 000/litre. In Indonesia, a well-established backyard-type nursery is used. This consists of a series of elevated canvas or concrete 1-2 tonnes tanks and similar stocking densities to those used in Taiwan Province of China are employed. In the Philippines, milkfish nurseries are integrated with grow-out facilities, where wild-caught or hatchery-reared fry are first acclimated into nursery compartments which comprise one third to one quarter of the total area of the brackish water pond. Fry are stocked at a density of up to 1 000/litre and are fed with a naturally-grown micro-benthic food known as 'lab-lab' which grows on the fertilized pond bottom. When natural food is becoming depleted, artificial feeds such as rice bran, corn bran, and stale bread or formulated feeds are provided.

During the past decade, much progress has been made, particularly in regard to milkfish propagation and the mass production of fry by private hatcheries, research institutions and government agencies. Instead of relying on wild-caught fry, milkfish farms in the Philippines, Taiwan Province of China and Indonesia now obtain the majority of their fry from hatcheries, mainly due to the significant shortage of wild-caught fry.

Shallow water culture is practiced mainly in Indonesia and the Philippines. Milkfish are traditionally cultured in shallow Brackish water ponds in which the growth of benthic algae is encouraged through inorganic or organic fertilization. Milkfish will survive on benthic algae alone only if the productivity of the algae exceeds the grazing rate of the fish; otherwise, supplemental commercial feeds are applied. The 'lab-lab' culture system in the Philippines is equivalent to shallow water culture in Taiwan Province of China. 'Lab-lab' is the term used in this country for the algal mat (and all micro-organisms associated with it) in the ongrowing ponds. Brackish water ponds in the Philippines were mostly excavated from 'nipa' and mangrove areas. Shallow water pond design generally consists of several nursery and production ponds with a typical area of 2 000 m^2 for nursery ponds and 4 ha for production (ongrowing) ponds. Typically, ponds have a depth of 30-40 cm and are provided with independent water supplies. The average yield of a typical integrated nursery, transition and shallow grow-out system that produces 3 crops a year is 800 kg/ha. Modified modular pond designs consisting of a series of grow-out compartments with a maximum of eight crops a year have been shown to increase yield to a high as 2 000 kg/ha.

Deep water culture was developed in the mid-1970s in response to the decline of profitability of shallow water culture, and the limited and increasing value of land and manpower resources. Deep-water ponds provide a more stable environment and extend the grow-out period into the winter season. Most deep-water milkfish ponds have been created by converting either shallow water ponds or freshwater ponds, with a depth of 2-3 m. Production from these systems has sharply increased in Taiwan Province of China.

Most milkfish ponds in the Philippines and Indonesia are of the extensive and semi-intensive type, with large shallow pond units, tidal water exchange, natural food, minimal use of fertilizer alternating with commercial feeds and other inputs, and low to medium stocking rates (50 000-100 000/ha). The Taiwanese method of production, on the other hand, employs intensive stocking densities (150 000-200 000/ha).

2. Indian scenario

Milkfish (*Chanos chanos*), is naturally present in Indian and Pacific Ocean. It is a national fish of Philippines where it is known as 'Bangus'. Milkfish is a tasty fish. It can tolerate wide range of salinity. Culture of milkfish in brackish water ponds and pens is an age-old and traditional practice in many tropical countries such as Philippines, Taiwan and Indonesia and Pacific island countries. It is also farmed in freshwater ponds, lakes, reservoirs and in marine cages in these countries. Global production is about 9 lakh metric ton during 2012. In 2014 Philippines, the leading producer of milkfish has produced 391,983 MT. In India, occurrence and traditional culture of milkfish has been reported in coastal waters, estuaries and brackishwater water bodies such as Chilika lake in Odisha, Pulicat lake in Andhra Pradesh etc since long back. It was a favorite fish of Mysore king Tipu Sultan in the 19th century and he used to procure it from brackishwater ponds from Kundapur in Karnataka. It is called as Paal Meen in Tamil, Pala Bontha and Tulli Chepa in Telugu, Poomeen in Malayalam, Hoomeenu in Kannada, Golsi in Goa and Seba khainga in Oriya. Its important characters such as herbivorous feeding habit, low disease occurrence, rapid growth, wide range of salinity tolerance, attractive appearance, longer shelf life in ice, good taste and texture and suitability as live bait in tuna industry, makes it a potential candidate for culture to revolutionize aquaculture growth.

3. Status of seed sources

Worldwide, Milkfish seeds were collected from coastal areas since beginning for brackishwater farming. This has led to decline in fry availability in nature presently. In 1970's Philippines developed hatchery technology for seed production of milkfish which has given an impetus for the milkfish farming. In India, wild caught seeds are collected in the months of March to June and September to December from coastal states of Tamil Nadu, Andhra Pradesh and Kerala etc. by traditional methods. Fry are more abundant during the new and full moon periods. In India, increasing trend of the milkfish farming still depends on the availability of seeds from wild resources which lacks good quality and sometime comes along with predatory fishes. Therefore, technology development of breeding, seed production and culture practices in brackishwater area is important for the development of milkfish production in India.

4. Farming system

4.1. Pen Culture:

Pen culture of milkfish was first introduced in the Philippines in 1979. Fish pen size ranges from 1 ha up to 50 ha. In this system, the milkfish feed mainly on plankton and also forage for food at the bottom. However, there are times that supplementary feeding may be required especially when stocked at higher densities or natural food becomes depleted. Stocking density is about 30,000 to 50,000 fingerlings per hectare which equals 1 fish per m3. The fish grow to market size (250-300 g) in 4 to 8 months with survival of 60-80% and yield from 4,000 kg/ha to as high as 10,000 kg/ha. The fish reach harvest size of 250-275 g in 4-5 months with a survival rate of 80-90% and production of 1.5-5 kg/m^2.

4.2. Milkfish Culture in Cages.

Milkfish cages may be installed in freshwater lakes, estuarine areas, and coastal marine waters. Cages may be square or rectangular using bamboo frames or G.I. frames with drum floats. More advanced design consists of high-impact polypropylene pipe frame which serve also as float. Feeding of complete formulated diet (27-31% protein) is essential from stocking of the fish to harvest. Small-sized fingerlings (5-10 grams) are initially stocked at higher density in cages with nets having small mesh size for 1-2 months before being transferred at desired density to grow-out cages. Stocking density depends on the carrying capacity of the cage and the environment. Typical stocking densities in floating and stationary cages are 10-40 pcs/m3 with a survival rate ranging from 70-90% and yield of 3-20 kg/m^3. Offshore cages can be stocked with 40-100 pcs/m^3 with a yield from 20-35 kg/m3 .Sizes at final harvest typically range from 350 to 500 grams. .

4.3. Polyculture:

Milkfish is reared with shrimps, mud crab, rabbitfish, seabass, tilapia, seaweeds, mollusks, and many other fish species either as primary or secondary crop. The polyculture of milkfish with shrimps or with crabs however, are the most popular and profitable. They compliment each other in terms of habitat and food requirements. Annual yield of milkfish as the primary stock when grown together with shrimp ranges from 1,200 to 1,800 kg/ha while annual shrimps production is from 100 to 200 kg/ha. On the other hand, about 550 kg per ha of milkfish and 1,500 kg/ha of crabs per crop can be attained using the polyculture method. Generally a minimum of 2 crops per year can be undertaken when milkfish is polycultured with either shrimps or crabs.

4.4. Extensive traditional farming

In India, traditional milkfish farming can be done in different brackishwater areas like Bheries in West Bengal, Chilika Lake in Odisha, Pokkali in Kerala and Ghazni in Karnataka & Goa. These traditional ponds are having water depth of 40-60 cm which is suitable to stock milkfish fingerlings of 7–10 cm body size with stocking density of 1000-1500 fingerling/acre/crop depending on the cropping pattern. 2 crops/year can be harvested in batch stocking cropping pattern. After every 15 days, partial harvesting can be done by using gill net in continuous stocking cropping pattern. Every partial harvest is followed by re-stocking with milkfish fingerlings. In extensive traditional farming, milkfish fingerlings feed on only natural food like Lab-lab (benthic algae) and Lumut (filamentous algae). No artificial feed is provided. Final harvest of 1.5 to 2.5 tons/hectare/year is achieved with lablab feeding whereas lumut feeding yields only 500- 600 kg/ha/year.

4.5. Intensive culture:

The intensive milkfish culture requires smaller (0.1-1 hectare) but deeper (1-2 m) grow-out pond, enormous capital investments, large working capital, and technical proficiency. Paddle wheel aerators, feeding devices and pump for water exchange assist to increase the natural primary productivity of pond. Milkfish fingerling of 7-15 cm body size with stocking density of 8,000–12,000 fingerlings/ha to highest density of 30,000 fingerlings/ ha can be stocked in ponds. Feeding with floating pellet (CP 24-28%, CF 3-4%) improve FCR. Daily feed ration should not exceed 1.5% of total biomass in a pond. After 3-4 months of culture, Milkfish (200-300 g) can be harvested with the help of dragnet or gill net. Production of 4–6 tons/ha/year to 12–15 tons/ha/year can be achieved after a culture period of 3 - 4 months. Mass mortality is a constant threat due to accumulations of toxic metabolites such as ammonia and sulfides, oxygen depletion, and diseases. Procedures in pond preparation, maintaining

4.6. Semi-intensive Culture:

This method is characterized by smaller pond size of 1 to 5 hectares, at least 1 meter depth of water, and an increased stocking rate of 8,000 to 12,000 fingerlings per hectare in the rearing pond. Water exchange is enhanced by widening the gate, provision of separate drain gate and using water pump. Oxygen supply is improved by providing paddlewheel aerators and maintaining good phytoplankton growth later in the growing period. Natural food, mainly lab-lab, is grown and used as food in the first 45 to 60 days of culture in the grow-out ponds

and commercial formulated diet with at least 27% protein is supplied thereafter. This method allows 2 to 3 crops and yields of up to 7.5 tons per ha per year.

5. Future Perspective

Milkfish culture has potential to revolutionize the brackishwater farming due to its low input cost, disease resistance, high profit and environment-friendly farming systems. The domestic market demand for milkfish consumption is very encouraging and gives hope for its ready acceptance. Milkfish also a preferred bait fish for tuna long lining and gives huge market potential as live bait. It also observed milkfish has ready ornamental potential due to its shiny appearance, V shaped caudal fin and agile swimming behavior. A complete technology package for seed production and farming of milkfish has been developed by the CIBA. Therefore, it is a great opportunity for the farmers/entrepreneurs to take up milkfish farming in India as income generation activity. However, scarcity of stock size seed (fingerlings/advanced fingerlings) in adequate quantity is restricting the expansion of large-scale farming of this species. Participation of private hatcheries for commercial scale seed production of milkfish is need of the hour to meet out the increasing seed demand from the farmers. CIBA is encouraging the stake holders to produce the fingerling seed in their farm site facility by obtaining the spawn/larvae and fry from CIBA. ICAR - CIBA has taken up initiatives and a Memorandum of understanding (MoU) was also signed with Raj hatcheries, Tajpur (West Bengal) to give impetus to the production of hatchery-produced seed from commercial hatchery in the near future.

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8

Asian Seabass *Lates calcarifer* - Breeding and culture

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1. Introduction

Asian seabass is an important food fish in marine, fresh and brackishwater in Indo-pacific region, most sought after candidate species for aquaculture in recent years and it has expanded as candidate species for cage culture and in the recirculating systems globally particularly in South East Asia, Australia and Saudi Arabia (Bhatia & Kungvankij, 1971).

Asian seabass *Lates calcarifer* is an euryhaline fish belongs to the family Centropomidae widely distributed in the Indo-West Pacific region, Arabian Gulf to China, Taiwan Province of China, Papua New Guinea and northern Australia between longitude 50°E to 160°W and 24° N, 25° S latitude. It is found throughout the northern part of Asia Southward, Queensland (Australia) West ward to East Africa (Copland and Grey1987).

Lates calcarifer is known as seabass in Asia and Barramundi in Australia (derived from the aboriginal word 'barramundi' meaning 'large scales') and it has also been variously called as 'giant perch', 'cock-up' 'anama' in Papua New Guinea, 'kakap' in Indonesia and Malaysia, 'bulgan' in the Philippines, 'bhetki' in India and generally as Asian seabass in the literature. In vernacular languages, Koduva or Kooral in Tamil, Narimeen or Kaalanji in Malayalam, Kurudi meen or Mudar menu in Kannada, Pandu koppa in Telugu, Jitada in Marathi.

2. Biology of Seabass

Asian seabass spends the growing phase of life in fresh or brackishwater, migrates to sea for breeding.

Seabass is protrandrous hermaphrodite means early stage upto 3 kgs male and converted to female after 3-4 kgs or age

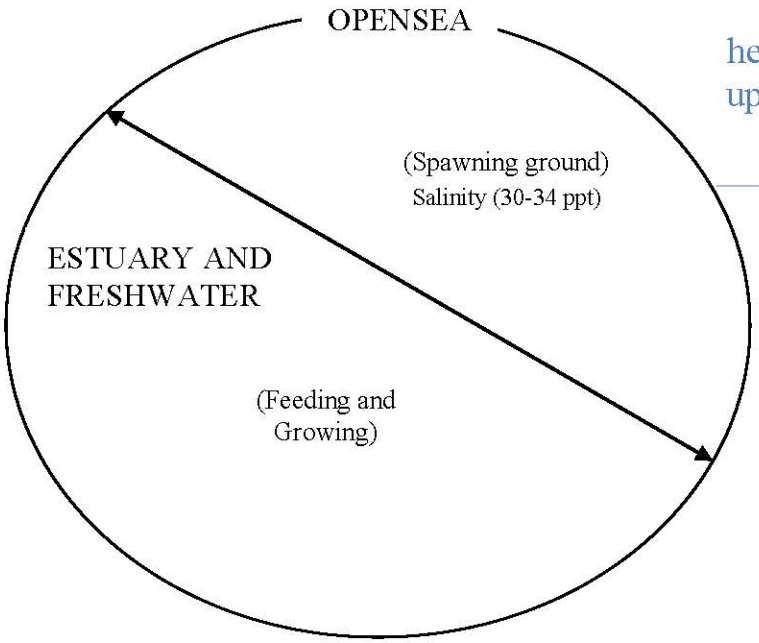

Fig. 1. Asian seabass *Lates calcarifer* Life cycle

In seabass, males attain maturity when they are above 2 kgs in the age of 2+ years and females generally attain maturity after 3 years when they are around 4 kgs in weight (Davis, 1982, Kuldeep,1991). In matured males milt will ooze out as thick milky white viscous fluid when the abdomen is gently pressed. In the case of females, in fully ripe ovary, the eggs will be separate occupying the entire body cavity (Kuldeep, 1991), seabass is protandrous hermaphrodite (Davis, 1982, Russel and Garrett, 1985), first male, then converting in to female after three to four years.

Seabass is a protracted batch spawner capable of spawning many times in a season. A female fish can spawn upto 7 million eggs (Ruangpanit, 1987). Toledo *et al* (1991) reported that a female weighing about 7 kg could naturally ovulate 8.9 million eggs in a season. Seabass naturally spawning in new moon or full moon to near quarter moon phase in Asia for at least 4-5 months.

3. Collection of broodstock

Broodstock fishes can be collected from wild conditions from sea and estuary, farm reared fishes from pond and cages. The healthy, fishes devoid of injury has to be selected, it should be transported carefully in truck with soft insulated material lined tanks/water tanker with provision of required oxygen. Transported fishes to be acclimatised with same water quality of the transport system and source water with flow through, fishes has to be quarantined for 7-10 days in isolated place not connected with hatchery, after examination of

fishes for any injury, bruises, parasites, after giving freshwater dip or prophylactic treatment with 100 ppm formalin, after screening for viral, bacteriological and pathogens, the conditioned procured fishes can be added to broodstock holding tanks.

Long distance transport by Truck with Oxygen bubbling

Broodstock procured from nearby wild catches

In captive land based broodstock development, the ideal holding tank is RCC tank, CIBA has successfully maintaining broodstock fishes in 100 tonn RCC tank (12 x 6 x 2 m) with provision of water inlet and draining. The fishes maintained @1kg/tonn biomass.

Broodstock fishes can also be maintained in net cages in sea/backwater/brackishwater pond with good water current, the net cages with depth of 2 m in the size of 5x5 or 10 x 10m, mesh size 8 t0 10 cm, stocking density 2kg/m^3. Recirculating Aquaculture System (RAS) facility may also feasible for maintenance of broodstock fishes, the fishes can be maintained @5kg/tonn in 10 tonn FRP tank with RAS facility, this system is advantageous, in small area can hold high biomass, early maturity, spontaneous spawning and year round breeding is achievable.

4. Broodstock Management

4.1 Water

Source of water for broodstock fishes are generally open sea water or bore well with salinity in the range of 25 to 30 ppt, The water quality parameters to be maintained in the broodstock tanks are as follows: Temperature - 28 - 32°C Salinity - 28 - 33 ppt PH - 7.0 to 8.2 Dissolved oxygen - more than 5 ppm, Ammonia - less than 0.1 ppm, Nitrite-N - less than 0.01 ppm, Phosphate - less than 10 - 20 mg/l, Suspended solids - less than 2 - 5 mg/l.

4.2 Diet

Broodstock fishes are the source of production of quality seed, feed plays main role in determination of spawning and successful larval rearing with good survival. Hence, high quality feed to be given for broodstock fishes, generally for broodstock fishes marine trash fishes such as Oil sardines, mackerel.

5. Spawning

Seabass fish spawning season in Indian sub continent prevails from April to September/October based on monsoon season. Smaller fishes below 3 kgs are males and above 4 kgs 3+ years are generally female. Males can be identified by oozing milt if gently press abdomen. Females with ova dia of above 420µm is selected through ovarian biopsy with inserting polyethylene cannula of 1mm dia in genital pore up to 3 to 5 cm. Sex ratio for breeding of seabass is 2 males for 1 female (1:2), female injected with LHRH-a hormone @70µg/kg per body weight and 35 µg/kg per body weight for males.

Hormone administered intramuscularly one inch below dorsal fin of the fish, injected fish released in spawning tanks with flow through and egg collection facility. Normally, in most cases, spawning is good if coincide with lunar cycles and injection in early morning in order to spawn after 30-36 hours in evening times. Fertilized eggs are transparent and in the size of 780 to 850 µm float on the surface of water, unfertilized eggs are white and settle on the bottom. Seabass releases 0.5 to 1.0 million per spawning, it is protractor batch spawner hence it may be allowed in spawning tank for subsequent spawning's.

Hormone injection

Spawners

6. Incubation and Hatching

Collected eggs rinsed with fresh seawater, stocked in incubation of 250 l capacity conical bottom tank with inlet and outlet and provision of aeration, stocking density normally 500 nos/l preferred and in case of heavy spawning maximum up to 2000nos/l can be stocked, mild aeration and flow through with same salinity seawater of spawning tank @ 3 to 5 litre per minutes to be provided depends upon stocking density, optimum temperature for normal hatching is 29°C to 30°C. Fertilized eggs undergoes different embryonic development up to 16-17 hours as follows

Hatchlings measures around 1.2 to 1.5 mm size, after two hours of hatching, the water flow through and aeration are stopped before collection of larvae for 15 to 20 minutes, gently swirled the water enables the unhatched eggs and egg shells settle at the bottom, the hatchlings float on the surface of the water. The tanks bottoms are drained or siphoned to remove debris, the larva are kept in incubation tank until it is collected for stocking or packing to some other rearing centres.

The larvae are collected gently with bowl or beaker are immersed on water surface allow enter larvae with water and released in basin or buckets. The random sample are taken from subsamples and counted for stocking or packing.

7. Larval Rearing

Larval rearing is the process involves period of hatchlings to produce metamorphosed final 'fish shape' seed to eat formulated diet. Seabass larvae are reared in hatchery up to 15 to 20 mm size (3 to 4 weeks) in larval rearing tanks. Seabass is highly carnivorus due to differential size growth small ones eat bigger forming 'shooters' which reduces survival rate ultimately.

7.1 Larval Rearing System

Larval rearing mostly undertaken in indoor conditions only, larval rearing can be done in tank system either in Reinforced Concrete tanks (RCC) or Fiber Glass Reinforced Plastic tanks (FRP) of 5 to 10 tonn capacity circular or rectangular tanks. Tanks are provided with water inlet and outlet in bottom as well as in top for flow through system, aeration.and provision for RAS in case of highly intensive larval rearing. Intensive larval rearing is normally undertaken in India with indoor green water system. Larval rearing tanks are 5 to 10

tonn capacity, stocking density of hatchlings is 30 to 40 nos/l, subsequently reduced to 10 to 15 nos after artemia feeding stage, later further reduced to 4 to 6 nos/l.

7.2 Larval Rearing Management

In larval rearing tank (LRT), water quality has to be maintained for successful production, the initial stocking are in 25 to 30 ppt filtered,uv treated water or dechlorinated water. LRT water can be exchanged periodically or in top up method addition of water everyday upto 12th day, in traditional method water is exchanged after 4th day upto 30% level through filter nets or tubs with mesh size of 200 to 400µm. The bottom of the LRT to be cleaned whenever debris observed particularly after 6th or 7th day, in top up method after 15th day. The ideal water quality parameters are Salinity 25 to 30 ppt upto 15th day, gradually decreased to low salinity afterwards, ammonia less than 1 ppm, pH 7.6 to 8.0 and other parameters are within permissible levels.

Seabass larval rearing feed regime starts with adding required algae and frequent addition of zooplankton for feeding larvae as scheduled. This larval rearing needs production of huge quantity of algae and rotifer in batch cultures, starting from indoor stock culture, scale up and mass culture of algae and rotifer tanks in 1:2 ratio. Feeding regime of the seabass larval rearing system are given below:

Day 1	•Algae (20-30 /10^3 cells/ml
Day 2	•Rotifer 1-2 nos/ml •Algae required quantity to maintain light green colour
3-5 days	•Rotifer 3 to 4 nos/ml •Algae
6 - 9 days	•Rotifer 5 to 6 nos/ml + Algae
9-10 days	•Rotifer 6 nos/ml •Artemia 0.5 to 1 nos/ml + Algae
10-15 days	•Rotifer 2 to 3 nos/ml •Artemia 2 to 4 nos/ml+ Algae
17-19th Day	•Weaning feed with 100 and 200µm @20% body weight •Artemia 4 nos/ml
20th to 30th day	•Weaning feed 300 to 500 µm @20% body weight, feed size preferred according to larval size

Grading of larvae are done periodically after introduction of artemia nauplii and formulated diet by observing the size difference in larval tanks, if larval size difference is more than 30% prevails in tank, larvae are collected and graded with series of fish graders with different pore size of 2, 4, 6, 8 and 10 mm respectively. The larval rearing tanks are to be cleaned atleast once in a week either during grading or periodically for hygienic rearing, prophyltectic treatment with 100 ppm formalin once in 15 days is advisable during weaning period.


Grading


Seabass fry


Graded seed


Fingerlings

8. Nursery rearing

Hapa nursery is the common practice adopted by small farmers in their grow out pond itself, it needs less space, insitu environment, involves less capital investment and can be increased or decreased depends upon the grow out system available.

Battery of cages in the size of 2 x 1 x 1m made up of nylon/polyethylene of mesh size ranging from 1 cm to 3 cm, the stocking density for hapa are minimum of 300 to 700/m^3, the hapa can be increased depends upon requirement with provision of aeration and quick water exchange or natural flow. Feeding as per the standard protocol described in tank nursery through feeding bowl provided in hapa or automatic feed dispenser, periodic manual grading and hapa cleaning are done for better survival. The survival rate is around 70 to 80%.


Hapa Nursery in Pond


Hapa Nursery in Lagoon

9. Grow out Culture

Grow out of fish culture is rearing of fingerlings to greater than of 20 to 40 cm for marketing. Grow out culture are undertaken in pond, cage and recirculating (RAS) system in fresh, brackish and marine water. Mainly pond culture is practiced in large extent followed by cage culture, RAS culture is on initial stage. Traditionally seabass culture practiced by farmers for centuries with wild seed, after ensuring the availability of hatchery produced uniform size, required quantity and weaned seed has revolutionized the seabass grow out culture in India. Seabass culture practiced as mono culture and polyculture in pond system, in cage and RAS system mono culture with formulated feed only.

9.1 Pond culture

Earthen pond is ideal for culture of seabass, pond should be located where a good water facility, easy transport and feed materials are readily available. Culture pond are constructed after proper soil and water test for suitability, pond designed and constructed in the size of 2000m^2 to 4000 m^2 in rectangular shape will be ideal, pond have the facility for inlet water opposite to that outlet with collection pit and sluice gate with gradual slope to drain the water quickly for collection and harvest, provision of electricity and freshwater facility is desirable.

Three tier system of culture can be followed in pond culture also, three tier culture includes, Nursery phase, pre grow out and grow out phase. Nursery rearing is already described in nursery section. For pre grow out and grow out culture there are two type of culture followed in seabass farming as follows:

1. Monoculture
2. Polyculture

9.1.1 Monoculture:

Culture of single species seabass in pond with supplementary feeding, the pond is filled with water of 1 to 1.5 m , stockable size fingerlings are released in the pond @ 1500 to 2500 per acre pond, feeding with trash fish or formulated pellet feed. Trash fish @20% of the body weight initially for the total biomass, gradually it can be decreased 15, 10, 8, 5, 3% according to the growth. Water quality are monitored regularly, water exchange is done to the extent of 30% regularly and according to the water quality parameters. If follow three tier method, the fingerlings grown up to 200 to 300 size is harvested and stocked in grow out

pond, in this method uniform growth will be achieved in same culture period for entire stock since we are segregating small and big ones facilitate feed for all fishes and uniform growth. Feeding with trash fish FCR will be 1:5 and for pellet feed it is around 1:1.3 to 1.6 are ideal.

9.1.2 Polyculture

Culture of seabass along with forage fish in pond is followed in polyculture method. In polyculture system, pond prepared for algal blooming followed by stocking of forage fish like Tilapia sp. @ 20000 nos per acre, meanwhile the seabass are grown in nursery phase for growing stockable size fingerlings, once the fingerlings are ready to stock and Tilapia stocked in grow out pond bred profilic, the seabass fingerlings are stocked @1500 to 2000nos per acre. In three tier method, the juveniles are collected after attaining 200-300 gms size and released in another grow out pond with already grown forage fish, this method facilitate the early growth of juveniles to marketable size and ensuring maximum growth of stocked seed.

9.2 Cage Culture

Cages are constructed in circular shape with GI pipe and low cauge HDPE pipes, cage strength designed according to the wave and water current situation in growing site, nets are fabricated using nylon nets in the mesh size ranges from 2 to 8 cm. Cages can be made to afloat with floating materials such as metal, bamboo, styrofoam or with empty drums. The juvenile fish in the size range of 80 to 200 gms are stocked @ 10 to 20 kg/m^3 depends on range of factors, there is no one to fit all stocking density in cages. Fishes are given with trash fish or pellet feed, trash fish initially @10% of body weight after attaining 500gms size reduced @5% body weight, pellet formulated feed made with more than 40% protein are used @20% of body weight initially and gradually reduced to 5%, the appropriate size pellet should given according to fish size. The FCR for trash fish is 1:5 or 6 and for pellet feed ranges from 1:1.4 to 1:1.8.

Three tier cage culture

9.3 Grow out in RAS

In recent times, seabass culture is undertaken in Recirculating Aquaculture System (RAS) tanks with heavy investment, in small area production of large quantity with establishment of RAS provided with sand filter, biofilter, drum filter, oxygenator in indoor system. Water exchange at the rate of 100 to 300% per hour, feed with pellet feed, stocking @30 to 80kg/m^3

10. Harvesting and Marketing

Stocked fish are harvested according to the size demand in market and consumer preference of the market area. Fish usually harvested after 8 to 12 months in the size of 1 to 1.5 kg average, partial harvest is advisable to remove big size fish enables small size grow to marketable size in culture period. In India, fish price are depends on festivals and holidays, normally seabass fetches good price ranges from Rs.400 to 600/-, small farmers can harvest fish periodically and sell to local people in premium price during demand, large quantity harvest are done after exploring market in metro cities major fish markets, export of seabass also done by fish processing unit provided adequate quantity are harvested for single consignment.

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9

Breeding and seed production of pearlspot, *Etroplus suratensis* (Bloch)

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Introduction

Class- Actinopterygii

Order- Perciformes

Family- Cichlidae

Genus- *Etroplus*

Species- *suratensis*

Pearlspot, *Etroplus suratensis,* is distributed in peninsular India and Sri Lanka. Its tolerance to wide range of salinities makes aquaculture of the species possible in both freshwaters and brackishwater bodies. Being omnivorous in nature, aquaculture of pearlspot is relatively simple, economical and especially suitable for small scale aquaculture for supporting livelihood of fish-farmers. Pearlspot is extensively farmed in brackishwaters of Kerala has shown productions upto 1t/ha when cultured with milkfish and mullets (George, 1971). Traditionally pearlspot has been cultured in pokkali fields of Kerala along with other brackishwater fishes. Pearlspot has chiefly been cultured by farmers as a component of polyculture in brackishwater systems. Small scale cage based aquaculture experiments showed that stocking pearlspot @ 200 nos m^{-3} in 2 m^3 net cages can give a production of 26 kg m^{-3} in 200-260 days using commercial feed (crude protein-20%) (Padmakumar, 2009). More recently with the support of the state fisheries department many farmers and Self-Help Groups (SHG's) in Kerala are involved in culture of pearlspot in small cage (2-3 m^3) and pond systems. However, one of the major limiting factors for expansion of pearlspot aquaculture is inadequate availability of seed for stocking in different culture systems.

Pearlspot exhibits a high degree of parental care and has very low fecundity as compared to other brackishwater fishes. Successful induced breeding of pearlspot has not

been reported. These are the main reasons which makes mass scale seed production of the fish challenging. Hence development of technologies which allow seed production at multiple locations in the form of backyard hatcheries or small scale seed production systems is important. However the fish is easier to breed compared to many other brackishwater fish and today different models in a range of systems are available or being tested, so that seed production can be conducted by entrepreneurs, Self- Help Groups or farmers themselves depending on their local resources.

The different methods of pearlspot seed production are discussed in this chapter.

i) Seed production of pearlspot in ponds

In pond based experimental trials conducted in CIBA, 50 brooders were stocked in ponds of area, 100 m^2 and depth, 1.2 m after systematic pond preparation (draining, liming, weed fish eradication, manuring for promoting phytoplankton bloom). Additional spawning surfaces as palmyrah leaves tied in bunches to fixed poles, coconut leaf petioles, coconut husks, bricks, pieces of asbestos sheets etc. were provided. Salinity ranged between 15- 30 ppt and transparency was higher than 0.5 m. Brooders were fed with artificial feeds prepared using groundnut oil cake, rice bran and fish meal fortified with vitamins and minerals @ 2.5%. On observing the presence of hatchings, manuring was done with cow dung @500kg ha^{-1} for enhancing of plankton production. Artificial feed (25-30 g) was also fed once early in the morning. A production of 3500 fry was observed in a year from 5 sets of breeding (Abraham and Sultana, 1995)

ii) Breeding of pearlspot in RCC tanks

Breeding of pearlspot fish has been standardized using 20 t RCC tanks provided with continuous water flow through. Half of the tank bottom is provided with a soil base (4 inches), earthen tiles for egg attachment and hide outs are provided within the tank. The tanks are stocked using mature pearlspot brooders. The brooders are selected based on the size, sex of the fish and bright coloration. A stocking density of 20 fish per tank at a male female ratio of 2:3 was used. The male and female fish are identified based on the appearance of the genital papillae. Pair formation and breeding occurs naturally. Eggs are deposited on the tiles and also on the walls of the tank. Fry are collected at regular monthly intervals by lowering the water level. A production of 1200-3500 seed per batch can be obtained regularly. Seeds produced from this tank breeding system are supplied to farmers and self-help groups.

iii) Seed production of pearlspot in small net cages/ hapas

In CIBA seed production of pearlspot has also been tried in small net cages (hapas) in the secondary discharge pond of fish hatchery. The pond has conditions of gentle water flow, salinity of approximately 25-30 ppt. Brooders are maintained in small cages on commercial fish feed. Small net cages/ hapas are being used (dimensions, 1x0.75x1 m). These are fixed by casurina poles. Clay soil in small plastic tubs is suspended at 0.5 m depth from a cross-fixed casurinapole. Just above the soil surface 1-2 ceramic tiles are suspended to facilitate egg attachment. Each cage was stocked with 3-4 brooders (TL> 150 mm) and preferably with one fish having reddish and enlarged genital papillae (indicative of readiness for breeding). Efforts at pair formation are usually observed a few hours after release of fish within cages. Gradually the dominant pair occupies the soil container and territorial defense centered on the plastic tub is also observed. The aggressive behavior of the fish increases towards breeding and thereafter continues as a defense for protecting eggs and larvae. Nest formation was observed in the course of the breeding behaviour by mouth siphoning of the soil which led to formation of small pits within the soil containers.

In the initial trials hatchlings were collected from pit nets in cage and subsequent larval rearing was practiced, following this method a seed production of 1000-1500 seed per cage could be observed. However, it is not always possible to observe the correct day of spawning especially if the water is not transparent. The majority of seed production trials were conducted by allowing seed to be reared within cage system with parental care. A production between 200-300 numbers of seed (avg. lenth: 28.11±1.49 mm; avg.wt: 0.66±0.04 g) were observed per cage within 2- 2.5 months. The advantage of the system is that it is simple, and adoptable by farmers especially cage farmers. It requires a minimum investment (Rs. 400-500/unit) and the labor involved is chiefly in the initial cage setting and final seed collection. This model was tested at with a pearlspot cage farmer at Ashtamudi lake, Kerala. Using a set of eight hapas the farmer produced approximately 3000 fry in six months.

iv) Seed production of pearlspot in tank based recirculation system

Breeding trials of pearlspot breeding were conducted in one ton rectangular plastic tanks provided with a continuous water flow using a biofilter facility. Each tank was provided with a small plastic tub filled with clay soil to facilitate breeding. Each breeding tank was stocked with 4 mature brooders (total length>160 mm) and fed with pellet feed twice a day @ 2-3 % body weight. Pairing in the tanks could be observed within 2-3 days of stocking and

the paired fish were observed to occupy the soil filled plastic container provided. The aggressive behaviour was observed to centre on this container and the breeding pair was seen to actively chase other approaching fishes from it. The aggressive behaviour increased towards the approach of breeding, even leading to the mortality of the remaining fish. Towards breeding the fish were observed to clean a small patch at the sides of the soil filled container. The first breeding was noticed after 24 days of stocking. The eggs were seen to be attached to the sides of the plastic container and the brood fish were observed to take turns in defending the eggs. The larval clutch observed at the bottom of the soil filled container were separated for larval rearing. One of the most promising results obtained was the production of 8000 larvae by a single pair stocked in one tank in six breedings at an average breeding interval of 17.6 ±1.12 and an average larval number per spawning of 1333.3±143.0. Pearlspot larvae collected from the tanks have been reared using alternate live feeds, rotifer *Brachionus plicatilis,* artemia nauplii and by co-feeding with commercial larval diets. On an average, by this method, a seed production of up to 1000 fry (2.0 cm size) per tank and per month can be obtained in 30 days period and annual total production of up to 12000 seed per tank per pair of pearlspot.

v) Breeding of pearlspot as single pairs in outdoor green water tanks using formulated maturation feed:

Though pearlspot is naturally a low fecund fish, it has a great potential for farming in varying salinities ranging from 0 to 30 ppt. Maximum reproductive potential any fish can be realized maximum with an optimum nutritional back-up and suitable rearing system. CIBA formulated a maturation diet to have balanced amino acids and fatty acids to meet the minimum requirements optimized for cichlid fishes. This maturation diet in combination with a more economical out-door green water rearing system was found to be optimum in exposing the maximum breeding performance in pearlspot. We have achieved repeated spawning and higher fry yield with single pairs. While average fry production per pair per spawning was around 2500, a highest fry yield recorded was 3480 per spawning. While average number of repeated spawning per year was around 4 times, a maximum of 8 repeated spawning was recorded. Average inter-spawning days ranged between 35 to 40 days. Highlight of this breeding model is, we can have a good control over the parental fish and their young ones produced. Further it is more economical in terms of water management and feed management for both parents as well as their young ones.

With a planned feeding strategies and rearing model we were able to close the life cycle of pearlspot by the age of 11 – 12 months in small one ton tanks itself. This is a good indication that, pearlspot could be a good candidate for genetic selection. Termination of parental care was found to be a key driver in inducing the pearlspot for recurring spawning. Hence, larval rearing in the absence of parental care would be a most crucial in successful seed production of pearlspot under captive conditions.

Conclusion

Pearlspot is emerging as an important food and ornamental brackishwater fish. In the recent years there has been an increased research focus on different aspects of pearlspot seed production. This has resulted in the emergence of alternate technologies which can be utilized by the stakeholders. We definitely see a promise that in the near future one of the main constraints of inadequate seed availability will be overcome and we may witness substantial increase in aquaculture production of pearlspot.

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10

Broodstock Development and Induced Breeding in Grey mullet (*Mugil cephalus*)

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Introduction

Taxonomy

Class Actinopterygii

Order Mugiliformes

Family Mugilidae

Genus Mugil

Species *Mugil cephalus*

The flathead grey mullet *Mugil cephalus* Linnaeus is the most widespread species of the family Mugilidae which comprises of 20 genera and 70 species. The species is known by the vernacular names of "Madavai" in Tamil, "Thirutha" in Malayalam, "Kathiparega" in Telugu, "Boi or Mangan" in Marathi, "Mala" in Telugu, "Gandhia or Boi" in Gujrati, "Bhangor" in Bengali. Grey mullet is a cosmopolitan species occurring in all the major oceans of the world. The species is discontinuously distributed mainly between the latitudes 42^0N and 42^0S in the freshwater, estuarine and marine habitats of the world. The species is recognised economically as an important food fish. The roe of the species is used to prepare "Bortaga cavier" a delicacy in Taiwan and Japan and hence referred to as "Grey gold" by the fishermen of the region. In India grey mullet has good market in all the coastal states fetching between Rs 300-400 per kg. Grey mullet is situated at the base of the food chain and feeds on detritus and benthic micro-algae thus playing its significant ecological role as a converter of primary productivity, particulate organic matter and detritus into quality fish protein. The significant market demand, tolerance to wide salinity ranges and ability to utilise the herbivorous and detrital food chain qualifies it as an excellent candidate species for aquaculture.

Globally in the year 2014, the total production of grey mullets was recorded at 1,51,794 t of which 12,360 t was contributed by aquaculture production. Thus aquaculture

made 8.14 % contribution to the total global production of the species, this is significantly low in comparison to the species like Asian seabass where 41.2 % contribution was made by aquaculture towards its total production. The reasons which make a fish an ideal candidate for aquaculture production are related to its seed availability, market value, growth rates, feeding niche and acceptance of artificial feed, adaptability to aquaculture systems, rearing environment and resistance to disease. Given its position in food chain and its good market value, grey mullets have enormous potential for contributing to a sustainable aquaculture model which is the need of the coming decades. It remain to be debated which of the factors whether the seed, feed or the market has to be strengthened to enable the species to realise its true aquaculture potential.

Currently, the largest contributors to the global aquaculture production of grey mullets is Egypt, followed by Republic of Korea, Italy, Taiwan province of China and Israel. Most of the global aquaculture production of grey mullet is reliant on wild seeds. In India, wild seed availability of grey mullets is during the July to August in the west coast around Puduvypu region and around October-November in the east coast of India around Kakinada. Aquaculture of grey mullets in our country is on a limited scale in few pockets. The culture is reliant on wild seed using natural productivity and supplementary feeding based on agro-bye-products. There is good scope of improving the fish production if consistent hatchery produced source is made available. It is in this context that broodstock development and standardisation of the induced breeding protocols assume significance.

Broodstock Maintenance

A quality broodstock forms the foundation stone of a breeding programme. Different facets that have to be taken care for maintaining a healthy broodstock.

Broodstock holding system- Lined ponds, tanks and raceways have been used conventionally for holding the broodstock. At MES, CIBA broodstock have been held in 100 t tons RCC tanks provided with continuous flow through of seawater pumped from a deep bore. Broodfish are maintained at a stocking density of less than 1 kg m^{-3}. Both these factors ensures optimal water quality conditions. The tanks are cleaned on alternate days. Relatively smaller sized fish are being maintained in pond system for developing future broodstock.

Procurement of brooder- Brooders of grey mullet can be procured from the wild or raised in ponds from early stages however the latter involves an investment of tremendous effort and time. A sizeable broodstock of grey mullets are desirable. Grey mullet seem to

exhibit a state of social hierarchy in which only a small fraction of dominant females mature (less than 20%) supressing the maturity of the con-specifics. This makes only a small number of mature females available for use during the induced breeding in the season.

Broodstock availability- The availability of grey mullet spawners and the peak breeding season is associated with the north east monsoon around October to January in Kovalam backwaters (Mohanraj, 1994). The spawning season of grey mullet from Chilka Lake is from September to December (Jhingran and Natarajan, 1969), Mahanadi estuary – September to December (Shetty et al., 1965), Goa around September to February (Das, 1978).

Environment and water quality

Photoperiod plays a key role in initiating gonadal development and stimulating oocyte growth. Water temperature is important for initiating vitellogenesis and regulates oocytes to functional maturity. Environmental cues especially falling temperatures triggers aggregation and subsequent spawning migration. Most of the records of spawning's are recorded in waters close to 20 ^{0}C mostly in deep offshore waters. The best results for attaining functional maturity for grey mullets are obtained at combination of temperatures and photoperiod of 21 ^{0}C and 6L/18D respectively. A salinity of 32 ppt is found desirable. Grey mullet females undergo vitellogenesis irrespective of salinity, however the rate of oocyte growth is slower in fresh waters as is the proportion of females successfully completing oogenesis. A salinity ranging from 13-35 ppt has been suggested as adequate for ovarian maturation. Being confined to freshwaters during the spawning season is considered a major obstruction to the natural progression of results leading to spawning in the wild (Tamaru et al., 1994).

Natural feed and formulated feed

Grey mullets are predominantly benthic foragers feeding mainly on detritus including particulate organic matter especially benthic microalgae as diatoms, foraminiferans, filamentous algae, protists, meiofauna and small invertebrates. Diatoms form 20-30% of the stomach contents of the fish indicative of its selective feeding habit. This is also indicated by the relatively long intestine of grey mullets to effectively breakdown diatoms in the diet. Hence in most of the pond based broodstock, maintenance a substantial quantity of periphyton substrates is desirable to allow a good surface area for the development of periphytic organisms.

Grey mullets are regarded as species having a relatively high fat content compared to other species, 4.9%. Broodstock of grey mullets have been maintained on formulated maturation feeds developed by CIBA. The world over broodstock of mullet have been maintained on feeds with a crude protein content ranging from 35- 40 % and a crude lipid content of 4-8 %. Being bottom feeders sinking pellets are used and the fish are fed at the rate of 3-5% twice daily. A feed rich in poly-unsaturated fatty acids and arachidonic acid, adequate vitamin e and carotenoids, astaxanthin are recommended for broodstock maturation and good larval quality.

Size at maturity and broodstock selection

Males of grey mullets mature between 250- 300 mm standard length while females mature at slightly larger size, 270- 350 mm. Males are reported to mature at approximately 3 years of size while females mature at 4 years. A minimum fork length of 310 mm or three years of age is suggested best for selection of broodstock.

Reproductive biology of grey mullets

Spawning grounds of grey mullets are located in the sea. Grey mullets are generally reported to spawn once a year and exhibit synchronous ovarian maturation. Fecundity is reported in the range if 1.2- 2.8 million for the species (Thompson 1983) and 0.5-2 million (Bester 2009) and 849 eggs per g body weight (Nash et al., 1974). The optimum temperature reported for egg development of grey mullet is 24 ^{0}C. The most suitable ova size for a successful induced spawning is 600 micro-metre *i.e.* when the oocyte is in the tertiary yolk globule stage, stage III. A fertilised egg is approximately 870 micro metre in size with an oil globule of approximately 350 micro metre. The reported incubation time is approximately 26 h at 25 ^{0}C and can range upto 48- 60 h based on the temperature conditions.

Parasites- Grey mullets can act as hosts for a number of parasites. Visual observation and periodic examination of the fish for parasites is conducted. Fish should be carefully observed daily for reduction in feed intake and swimming activity as signs of parasitic infection. Periodic chemical treatment on a monthly basis is done using 100 mg L^{-1} of formalin for 45 minutes. Infections of external crustacean parasite *Caligus* spp. and *Lernanthropus* spp. have been reported in the grey mullet broodstock maintained at MES, CIBA.

Hatchery production- Global status

The history of induced breeding of grey mullet dates back to the 1960's in the pioneering work of Tang (1964). Full scale commercial hatchery production of grey mullet is not yet common. Induced spawning is achieved on an experimental and semi-continuous basis at Hawaii, United States of America and Taiwan province of China. Few of the steps followed are outlined. Egypt, the largest producer of cultured grey mullet (over 90 % of the global production) has one experimental mullet hatchery producing few lakh fry annually and largely depends on wild collection of grey mullet fry for aquaculture. Italy is another major producer of cultured grey mullet. Unlike Egypt, most of the cultured fry in Italy originates from hatchery produced mullet fry.

Maturity assessment

Maturity assessment of the broodfish is conducted with the approach of the spawning season. The fish after being caught carefully and anaesthetised in 30 ppm 2-phenoxyethanol. These fish are cannulated to assess the ova diameter and the condition of the eggs. Reports suggest an ova diameter of 600 micro-m to be optimum for successful induced spawning.

Induction of maturity

For advancing maturity, CIBA uses hormone based pellets of LHRHa. A 200 micro-g LHRHa pellet is suggested for implantation in the dorsal musculature of the fish (Tamaru et al., 1989) for advancing maturity of the female of grey mullets. Use of a combination of LHRHa and testosterone pellets have been shown to result in accelerated oocyte growth. For males silastic implants containing 17-α-methyl-testosterone containing 10 mg 17- α-methyl-testosterone has been found effective for 10 months in inducing testicular maturity (Lee, 1992).

Induced breeding and larval rearing

On obtaining mature fish with ova diameter exceeding 600 micro-m, a priming dose of 20 mg kg^{-1} of carp pituitary homogenate and a resolving dose of LHRHa, 200 micro-g per kg^{-1} is considered as the best combination for induced breeding via intra-muscular injections. The fish are kept at a ratio of 2-3 males: 1 female for breeding. The fish are reported to spawn 12- 14 h of receiving the resolving dose. The eggs are incubated at around 500 no L^{-1} in aerated tanks. High salinities of above 35 ppt are recommended for incubation of eggs. At 26^0C an incubation time of 28 h is reported, based on the temperature incubation period of

48- 60 h is also reported. Larvae of grey mullet begin feeding on the third day when they are provided algae and rotifers. Feeding of artemia nauplii is initiated on the seventh day.

Other inducing agents used successfully in different studies

Partially purified salmon gonadotropin, SG-G100 has been recommended at doses between 12-21 micro-g per kg body weight in inverse proportion to the egg diameter ranging above 600-700 micro-m. One third of the total dose is given initially followed by the remaining two-thirds after 48 h.HCG- Priming dose of 20000 IU per kg followed by a resolving dose of 40000 IU per g after 24 h in fish with oocyte dia of 600 mcro-m (Kuo et al., 1973) LHRHa- 300- 400 micro-g per kg body weight, one third as priming dose and two third as resolving dose after 24 h (Lee et al., 1987).Many successful combinations were tried by Lee et al., (1988). A priming dose of HCG at 5000IU and a resolving dose of LHRHa at 200 micro-g per kg resulted in 100% spawning rate, so also an LHRHa priming dose of 200 micro-g per and a resolving dose of 20 mg per kg. However the best fertilisation rate of 66-86% was reported was using a combination of CPE and LHRHa at 20 mg kg^{-1} and 200 micro-g per kg respectively. Priming dose- 20-70 mg CPE or 10,000 IU HCG and a resolving dose of 200 micro-g LHRHa, ova dia- 570-580 micro-g was reported for successful spawning and fertilisation by Gharabawy and Assem (2006).

In grey mullets strong dopaminergic control is reported, hence more recently, GnRHa- priming dose- 10 micro-g per kg+ a dopamine antagonist- metoclopramide, 15 micro-g per kg and resolving dose- 20 micro g per kg+ a dopamine antagonist-metoclopramide, 15 micro-g per kg were given 22.5 h apart for successful spawning (Aizen et al., 2005).

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11

Captive breeding and seed production of the brackishwater ornamental fish silver moony, *Monodactylus argenteus* (Linnaeus, 1758)

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1. Introduction

Silver moony, Monodactylus argenteus (Linnaeus, 1758) belonging to the family Monodactylidae (Order: Perciformes) is a brackishwater species of high demand in the ornamental fish industry. This species thrives in broad habitats, comprising the open ocean, brackishwater, and freshwater (Kuiter & Tonozuka, 2001). They are widely distributed in the tropical Indo- Pacific, from East Africa, South Africa, Mozambique Channel, Madagascar and Mascarenes east to the Caroline Islands, Mariana Islands, Red Sea to Australia, Sumoa, Southern Ryukyu Islands and a single record at Jubail, Saudi Arabia (Jawad, 2013; Azeroual et al., 2017). The genus Monodactylus includes four species of which M. argenteus is the most common species in the brackishwaters of India. It has been reported from the brackishwater regions of the coastal states of Kerala, Maharashtra, Tamil Nadu and West Bengal. The juvenile of silver moony are collected during the January-April months especially from the coast of Tamil Nadu for the aquarium trade.

They are commonly known as 'silver moony' or 'monos' or 'diamond fish' because of their attractive body color and shape. It has a silver shaded body and snout with two vertical black stripes on the front, one running through the large eyes and other just behind it through the operculum. There is a light yellow tint on dorsal and anal with a black outline on the anal fin. The caudal fin is colored light yellow. Pelvic fins are rudimentary in the juvenile and adult stage. Juveniles have more yellow coloration and orange tint on the dorsal fin. Vertical black bands on the snout of the juveniles are more prominent and thick compared to the adults. They are usually seen in schools and aggregations with a preference to occupy, around and under the floating or sunken logs, branches, and mangrove roots (Kottelat, 2001; Allen, Midgley, & Allen, 2002).

Silver moony is an omnivore; feeding on plankton, smaller aquatic animals (decapods) and detritus in nature (Yeragi, Yeragi, & Shaji, 2014) and quickly adapt to artificial feed in culture. M. argenteus prefers to move in schools in natural habitat and aquariums. Its schooling behaviour, tolerance to sudden fluctuation in salinity, high market

price, and compatibility with other species (marine, brackish and freshwater) in aquariums makes it popular among all types of aquarium hobbyists. The rise in demand and seasonal availability of silver moony led to over-exploitation of natural stock because the entire ornamental fish trade of this species is based on the wild collection. In order to address this issue, ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA) has developed the hatchery technology of this species, with a production capacity of over 1 lakh seeds per year. The effective way to reduce pressure on the wild stock is to develop hatchery based seed production technology.

Figure 1. Silver moony (*M. argenteus*)

2. Broodstock development and gonadal assessment

Broodstock can develop in RAS systems, cages, happas and ponds. In net cages the stocking density can be maintained at 15 m^{-3}, and fishes can feed twice daily at the rate of 3-5% of wet body weight with a broodstock feed (2 mm, crude protein: 52 %, crude lipid: 10%) formulated at ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA). Vitamin C @ 100 mg kg^{-1} and Vitamin E @ 350 mg kg^{-1} should supplement once in a week, along with the formulated feed. There will be noo specific external features of sexual dimorphism present in silver moony and sex determination is only possible through gonadal biopsy. Monthly sampling is required to assess the gonadal development. Female gonadal biopsy can carry out, using a catheter of 1.70 mm diameter (FEEDY®, Romsons, FG-05). Maturity in males can assess by the presence of oozing milt on applying gentle pressure to the abdomen.

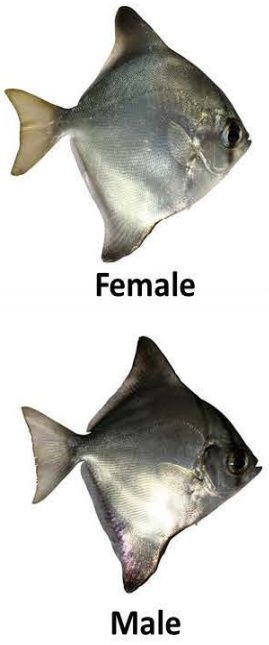

Figure 2. a) Broodstock holding facility (net cages 2x1x1m) of silver moony at Muttukadu Experimental Station of CIBA; b) and c) female and male broodstock of *M. argenteus*

3. Breeding system and spawning induction

Fibre re-inforced plastic (FRP) tanks (capacity, 500 L) are suitable for breeding. Seawater with a salinity of 30 to 35 ppt and dissolved oxygen > 5 ppm can be use for the breeding trials. The tank should provide with gentle aeration. Females with average mature oocyte diameter > 500 µm ($n \geq 30$) can select for breeding trials. The selection of males is made based on the presence of substantial amount of milt on applying gentle pressure on abdomen. Sex ratio can be maintain at the rate of one female and two males (sex ratio – 1 : 2) and both female and male should administer with a single dose of LHRHa (SIGMA, USA, L4513-1MG) at the rate of 1 mg kg^{-1} and 0.5 mg kg^{-1} wet body weight, respectively, through intramuscular injection. After injection, the female and males can be released in to the breeding tank.

4. Spawning, egg collection and incubation

Spontaneous spawning and fertilization will be observed after a latency period of 36 to 40 h. After spawning, fertilized eggs will be floating in the spawning tank. The spawned fishes will attain oocyte maturation within a period of 10 -14 days after the hormonal inducement. Fertilized eggs (830 to 900 µm) will be transparent, pelagic, non-adhesive with single oil globule (233 ± 6.31 µm) whereas unfertilized eggs will opaque and settled at the bottom. The average fecundity is 58.28 ± 5.18 g^{-1} wet body weight. The spawned eggs can

collected from breeding tanks using a hand net (100 µm mesh) and treat with 20 ppm iodophor for 10 min. Eggs can stock in 200 L black colored cylindroconical FRP tanks for incubation with central aeration to keep eggs in suspension. The total number of eggs in spawning tanks can quantify by taking five random samples of 50 mL and counted the number of eggs in each sample, and the average was taken. The fertilization rate can quantify in the same method to that used for estimation of the total number of eggs. The total number of fertilized eggs is divided by the total number of eggs spawned and multiplied by 100 to quantify the fertilization rate.

5. Hatching and larval rearing

Larval rearing can carry out in circular FRP tanks (capacity, 500 L). Tanks should provide with four air stones in a square pattern (diagonal distance between two air stones was 50 cm) with mild aeration. After hatching, the hatchlings can stock in the larval rearing tanks at the rate of 5 L^{-1} in triplicate from each breeding trials. Green water technique is suitable for larval rearing using microalgae, *Nanochloropsis oculata* at the rate of 100-200 × 10^3 cell mL^{-1}. Feed regime can consisted of live feeds; rotifer, and *Artemia* nauplii and artificial feed. The physicochemical parameters, such as temperature, salinity, total ammonia nitrogen (TAN), dissolved oxygen, nitrate, and nitrite should monitor daily and maintain to an optimum level.

On three days post hatching (dph), the exogenous feeding will start. From 5 dph onwards, the rotifer density should increase to 15 to 20 mL^{-1} till the 13 dph. From 14 dph onwards, the rotifer density can gradually reduce, and at the same time, co-feeding with *Artemia* nauplii can start. An artificial feed can provid from 19 dph for weaning.

6. Larval growth and metamorphosis

The newly hatched larvae will measure 1630 to 1680 µm in total length with a prolate spheroid shaped yolk sac and oil globule (Figure 5b). Newly hatched larvae remained near the surface water without much movement and there will be no mouth opening at the time of hatching. By 48 h, mouth will be opened with a mouth gap of 300 to 320 µm. Eyes will pigmented, and the pectoral fin is forms with visible dark pigmentation on the abdomen. By 72 h, exogenous feeding will start when the yolk sac is completely resorbed, and the larvae will measure an average length of 2100 to 2200 µm (Figure 5c). Pelvic fin will start to extend horizontally, and the larvae become free-swimming on 4 dph. On 5 dph, the larvae will measure 2700 ± 2750 µm with a prominent pelvic fin (Figure 5d), and larvae will start active swimming and feeding.

Notochord flexion and caudal fin ray formations will observed between 7 and 8 dph. On 13 dph, the body of the larvae become laterally compressed compared to the earlier larval stages with an average length of 6200 to 6260 µm and characterized by more intense body pigmentation, whereas, caudal part of the notochord will be without pigmentation. Active feeding migration begin by the 16 dph, and the larvae move in a circle in the larval rearing tanks. On 23 dph, metamorphosis will be start; squamation and intense pigmentation of the body begin changing from dark brown to golden yellow color (Figure 5f). The body become more laterally compressed, and two vertical black bands start forming in the snouts region. By 27 dph 50 % of the larvae will metamorphose into juveniles and characterize by a golden yellow body color with two vertical black stripes adorning the front, one running through large eyes and other just behind it through the gill cover and light yellow tint with a black outline on dorsal and anal resembling the adult fish. By 32 dph, 100 % larvae will transform to juvenile and could be reared to marketable size (4 cm) in 52 days post hatching.

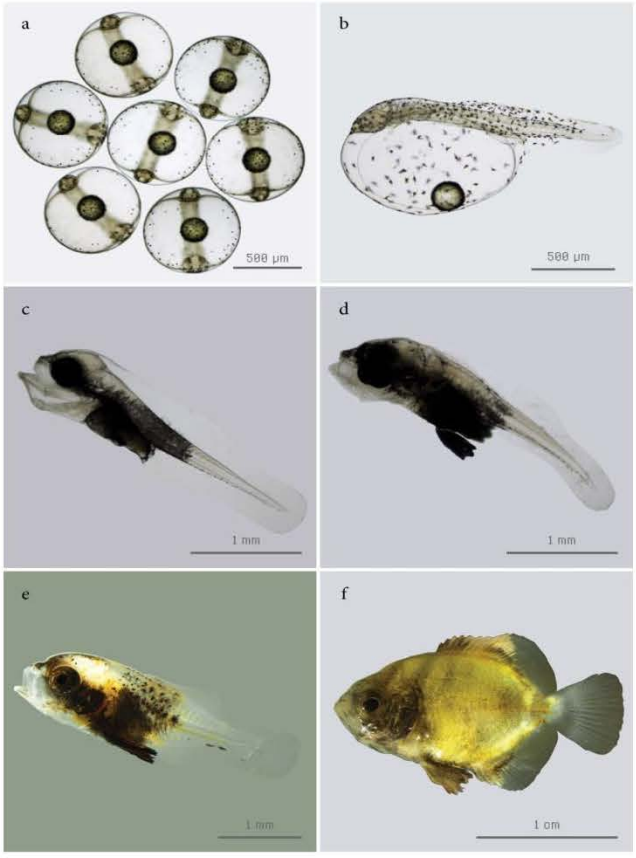

Figure 4. Fertilized eggs and larval development of silver moony under captivity. a) fertilized eggs with neurula stage; b) newly hatched larvae (0 dph); c) larvae with slender and pigmented body, eyes, open mouth and pectoral fin (3 dph); d) larvae with well-developed eye and pelvic fin; e) post flexion larvae with increased pigmentation on dorsal and abdominal regions (9 dph); f) metamorphosis of larvae to juvenile, body depth increased and body became denser with scales and golden yellow colouration. (dph, days post hatch)

Figure 5. Metamorphosed juveniles of *M. argenteus* (32 days old)

7. Technology transfer

The brackishwater ornamental fish rearing can be adopted as a backyard production system. The cost of maintenance and production of brackishwater ornamental fishes are less compared to the marine ornamental fishes. As an initiative for promotion of brackishwater ornamental trade and seed production CIBA has signed an MOU with ornamental fish farmer from Kerala to whom the first hatchery produced silver moony seed was supplied for nursery rearing and trade. Now the complete technology for breeding and seed production of silver moony is ready for transfer to the farmers, Self Help Groups and stakeholders.

Way forward

Present technology indicated that silver moony larvae could be reared to marketable size in 52 days with proper feeding and water quality management. Increasing demand for silver moony in the ornamental industry imparts more pressure on wild stock. Therefore, the present finding on the development of seed production of silver moony will pave the way for the transition of ornamental fish trade of this species from that based on the exploitation of wild-caught stocks to sustainable captive seed production systems.

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Innovative Farming Technologies of Brackishwater Fishes

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1. Introduction

Coastal aquaculture is a traditional practice in India. In the low-lying fields of Kerala (*Pokkali*), West Bengal (*Bheries*), Odisha (*Gheries*), Goa (*Khazans*) and Karnataka (*Kharlands*) which experience influx of saline water, traditional farming of fish/shrimp has been practiced. The practice includes allowing entry of juveniles of fish/shrimp in the fields and letting them to grow, applying supplementary feeding sometimes, facilitating tidal water exchange and harvesting periodically at 3-4 months. With the improvement of technologies and realizing the importance of aquaculture, these practices were improved with the supplementary stocking and water quality management resulting in moderate to higher production. The technology improvement made in the aquaculture sector has opened new areas for the scientific farming which is called as semi-intensive and intensive farming following all the protocols for farming with production as high as 10 ton/ha per culture period of 4-5 months for mainly shrimp and brackishwater fish like seabass production of 3 to 4 ton/ha/crop in the coastal area. Polyculture of fish and shrimp with different stocking patterns has yielded a productivity of 3 ton/ha/crop in South 24 Paraganas of West Bengal. In addition to that, farmers have achieved a production of 3.5 ton/ha/crop of milkfish when it is practiced in monoculture system in West Bengal and in Andhra Pradesh. Green water technology in brackishwater aquaculture has been standardized and farmers started adopting the same in different costal districts of Tamil Nadu. Phenomenal growth in Pacific white-leg shrimp, *Penaeus vannamei* farming has been occurred in the recent past. The technology advancement helped in the establishment of more than 390 shrimp and 1 crab hatcheries. The coastal aquaculture witnessed a rapid growth during 1980s and in the beginning of 1990s. But the shrimp aquaculture sector witnessed severe setbacks from the later part of 1990s due to socio-economic, environmental issues coupled with the outbreaks of uncontrollable

diseases. The major reasons attributed to this are the unregulated development and unforeseen disease outbreaks. The sole dependency on single species tiger shrimp, *Penaeus monodon* in coastal aquaculture has been switching to *P. vannamei* farming since last few years and this had pronounced impact on the coastal aquaculture sector questioning its sustainability. Therefore, diversification of culture systems involving brackishwater fishes is the need of hour. Compared to shrimp farming practices, brackishwater fish culture systems are less risky and environmentally sustainable.

2. Species diversification: a major step towards sustainable brackishwater aquaculture

The estimated potential area under coastal aquaculture is 1.2 million ha, of which only around 15% area has been brought under the culture. Considering the production potential of the sector, its production is projected to grow by four-fold by 2025 from the present at 0.84 million tons. Development of Indian coastal aquaculture in the country was driven by the technologies for seed production of tiger shrimp, *P. monodon* and the white shrimp, *Penaeus indicus*. Disease outbreaks due to WSSV during the last 20-25 years have acutely affected shrimp farming in the country and in other continents. With the diagnostic kits developed for detecting WSSV, PCR-tested seed is available all over the country. Supplementary feeding is the most important management measure in commercial shrimp farming. Commercial shrimp farming in India largely involves use of formulated pellet feed, constituting a significant share of the input expenditure. While bulk of the feed used was imported from Southeast Asian countries till a decade back, it is at present mainly produced in the country. Unavailability of quality ingredients, especially the fish meal, has been a major constraint faced by these industries, requiring import. The high price of the commercial feed, however, is forcing the small-scale farmers to resort to farm-made feeds. For the sustainable eco-friendly aquaculture practice, diversification to other species is considered as one of the important steps. Fishes like Asian seabass (*Lates calcarifer)*, grouper (*Epinephelus tauvina)*, snappers (*Lutjanus* spp.), which are high value carnivorous fishes and grey mullet (*Mugil cephalus)*, milkfish (*Chanos chanos)*, pearlspot (*Etroplus suratensis)*, rabbit fish (*Siganus* spp.), orange chromide (*Etroplus maculatus*) which are herbivorous/omnivorous suitable for farming in the coastal eco-system are available. The species like cobia (*Rachycentron canadum*), silver pomfret (*Pampus argenteus*) and pampano (*Trachinotus carolinus*) are being considered as candidate species for farming. Efforts have been made to develop comprehensive technology packages for seed production under controlled conditions and

farming of these candidate species. Technologies have been developed elsewhere in the world for several brackishwater and marine finfishes. In Indian scenario, the successful technology has been developed for the year round seed production of Asian seabass, *L. calcarifer* under controlled conditions and farming by the Central Institute of Brackishwater Aquaculture. The institute has also accomplished controlled breeding of milkfish, *C. chanos*, grey mullet, *M. cephalus*, pearlspot, *E. suratensis* and long whiskers catfish, *Mystus gulio*. In addition, a new avenue has been made by the successful breeding and seed production of ornamental fishes, spotted scat, *Scatophagus argus*, crescent perch (*Terapon jarbua*), orange chromide, *E. maculatus* and silver moonyfish, *Monodactyus argenteus*. Marine seaweeds have been a new area for brackishwtaer farming in different costal states of India. Successful demonstration of seabass farming has been conducted in all the coastal states. High export prices of crabs have made fattening of species like *Scylla serrata* and *S. olivacea* as a remunerative farming practice.

3. Seabass grow-out systems in ponds

3.1. Traditional grow-out practices

Seabass is cultured in ponds traditionally as an extensive type culture throughout the areas in the Indo-pacific region where seabass is distributed. Low-lying excavated ponds are stocked whenever the seabass juveniles are available in the wild seed collection centers (For e.g. April-June in West Bengal, May-August in Andhra Pradesh, Sept-Nov in Tamil Nadu, May-July in Kerala and June-July in Maharashtra). Juveniles of assorted size seabass are collected and introduced into the traditional ponds which will be already with some species of fish, shrimps and prawns. These ponds will have the water source from adjoining brackishwater or freshwater canals, or from monsoon flood. The juvenile seabass introduced in the pond will prey upon the available fish or shrimp juveniles as much as available and grow. Since seabass by nature is a species with differential growth, on introduction into the pond at times of food scarcity, the larger may resort to feed upon the smaller ones reducing the number. Seabass are allowed to grow for 6-7 months of culture period till such time water level is available in these ponds and then harvested. At the time of harvesting there will be large fish of 4 to 5 kg as well as very small fish. In this manner, production up to 2 ton/ha/7-8 months has been obtained depending upon the number and size of the fish entered/introduced into the pond and the feed available in the pond.

However, this practice is highly unorganized and without any guarantee on production or return for the aquaculturists. With advances in the technology in the production of seed under captivity assuring the supply of uniform sized seed for stocking and quality feed for feeding, the seabass culture is done in South East Asian countries and Australia in more organized manner. The major problem in the development of seabass aquaculture in India is the unavailability of seed in adequate quantity and in time and quality feed for nursery rearing and grow-out culture. The former has been overcome and the technology package for the seed production of seabass under controlled conditions is available. The suitable feed for the culture of seabass has been developed. The seed production technology developed by CIBA has already been commercialized and the feed technology (CIBA Bhetki AHAAR) is ready for commercialization. These technological improvements in the seabass culture have motivated the farmers to select seabass as a candidate species for aquaculture. Farmers have been adopting improved farming practices in seabass culture.

3.2. Improved seabass grow-out practices

The traditional culture method is improved with stocking of uniform sized seed at specific density and fed with low cost trash fishes/formulated feed of required quantity. Water quality is maintained with exchange periodically. Fish are allowed to grow to marketable size, harvested and marketed for high unit price. Seabass culture can be done in a more organized manner as a small-scale/large scale aquaculture in brackishwater and freshwater pond cages. This practice was further demonstrated in Public Private Partnership mode in three different costal states of India. Successful crops have been demonstrated in Andhra Pradesh, Tamil Nadu and Maharashtra.

3.2.1. Cage culture of seabass

Fish culture in cages has been identified as one of the eco-friendly at the same time intensive culture practices for increasing fish production. Cages can be installed in an open sea or in coastal area. The open sea cage is yet to be developed in many countries where seabass is cultured but coastal cage culture is an established household activity in the South East Asian countries. There are abundant potential in India also for cage culture in the lagoons, protected coastal areas, estuaries and creeks. Since cage culture of seabass has been proved to be a technically feasible and viable proposition, this can be taken up in a large scale in suitable areas.

Cage culture system allows high stocking density and assures high survival rate. It is natural and eco-friendly and can be adopted to any scale. Feeding can be controlled and cages can be easily managed. Harvesting is not expensive. Even in areas, where the topography of the bottom is unsuitable for pond construction, cage can be installed. Diseases can be easily monitored. Fish in cages can be harvested as per the requirement of the consumers, which will fetch high unit price. Above all, cage culture has got low capital input and operating costs are minimal. Cages can be relocated whenever necessary to avoid any unfavorable condition. In India, Rajiv Gandhi Centre for Aquaculture has successfully demonstrated the pond based cage farming of seabass.

In the cages, fish can be stocked @25-30 no./m^3 initially when they are in the size of 10-15 g. As they grow, after 2-3 months culture, when they are around 100-150 g, density has to be reduced to 10-12 no./m^3 for space. Cage culture is normally done in two phases- till they attain 100-150 g size in 2-3 months and afterwards till they attain 600-800 g in 5 months.

Fish in the cage can be fed with either extruded pellets or with low cost fishes as per the availability and cost. Floating pellets have advantages of procurement, storage and feeding. Huge quantity of low cost fishes are landed in the commercial landings in the coastal areas which fetch around Rs.10-15/kg only and can be used as feed for seabass culture. Low cost fish like tilapia available in freshwater and brackishwater also serves as feed for seabass in ponds and in many cage culture operations. The rate of feeding can be maintained around 20% initially and reduced to 10 and 5% gradually in the case of trash fish feeding and in the pellet feeding, the feeding rate can be around 5% initially and gradually reduced to 2-3% at later stage. In the feeding of low cost fish, FCR works out around 6 or 7 (i.e., 7 kg of cheaper fishes has to be given for one kg of seabass). In the case of pellet feeding, FCR is claimed to be around 1 to 1.2 in Australia. However, the cost effectiveness of the pellet feeding for seabass in grow-out culture has to be tested.

Under cage culture, since seabass can be intensively stocked and properly managed, the production will be high. Frequently culling and maintenance of uniform sized fish in the cages will ensure uniform growth and high production. Production of 6-8 kg/m^3 is possible in the cages, under normal maintenance and production as high as 20-25 kg/m^3 is obtained in intensive cage management in the culture of seabass.

3.2.2. Integration of cage culture of seabass with shrimp culture

If seabass can be weaned to feed on floating pellets, because of their addictive nature to selective feed, they will not resort to prey upon shrimp as normally experienced in shrimp culture ponds. If the water depth can be maintained around 1.5-2.0 m, in a pond, cages can be installed in the shrimp culture pond itself and seabass seed weaned to feed on floating pellets can be stocked in the cages and reared. In this way, seabass culture will be a complimentary to shrimp culture.

4. Monoculture of grey mullet, *Mugil cephalus*

Grey mullet can be farmed in monoculture ponds. The pond for monoculture is prepared first, following eradication of unwanted organisms and application of manures and fertilizers. Advanced fingerlings of >50 g size are stocked at 10,000 no./ha. Fish are fed with supplementary feed. In an 8-month culture, fish become 500-800 g with total production of 3-4 ton/ha.

5. Farming of milkfish, *Chanos chanos*

Milkfish, *C. chanos* is an economically important and widely cultured food fish of south-east Asia and has distribution in the tropical and subtropical areas of the Indo-west Pacific Ocean. ICAR-CIBA has been successful in captive breeding and seed production of this species. Milkfish is a suitable species for low input based culture system easily adoptable by small and traditional farmers.

5.1. Culture pond condition

- Depth: 1-1.5 m
- Salinity: 10-30 ppt
- Temperature: 16-30°C
- pH: 7.5-8.5
- Dissolved oxygen: 3.5-5 ppm
- Soil type: silty clayey loam

5.2. Culture method

- Pond fertilization: Fortnight application of cattle manure- 500 kg/ha or mustard cake- 200 kg/ha, urea and SSP- 20 kg/ha each.
- Periphyton substrate: White nylon net fixed vertically covering 20% of pond surface area.
- Stocking: milkfish fingerlings- 10000-15000 no./ha.

- Feeding: Pellet feed @ 2-3% of body weight.
- Growth: In 6 months, 400-500 g.
- Survival: 90%
- Production: 3.0-4.5 ton/ha

5.3. Economics of milkfish culture

- Operational cost: Rs. 2.6 lakh/ ha
- Total return: Rs. 4.7 lakh/ha
- Net return: Rs. 2.1 lakh/ha
- Benefit cost ratio: 1.81
- Profitability can be improved by stocking bigger size nursery reared fish seeds.

5.4. Multiple stocking and multiple harvesting (MSMH) model of milkfish farming

For small farmers return from farming at regular intervals is most desirable to meet up their daily needs. In this context, MSMH model of milkfish culture could be suitable to these farmers. Moreover, this method can augment productivity of a small pond by many folds.

Two consecutive trials were conducted with two stocking densities (7500 and 15000/ha) as treatments. Milkfish fingerlings (6-10 g) were stocked and reared in fertilized ponds (500 m^2) provided with a formulated feed (CP 30%) @3-5% of body weight daily. After 100 days, harvesting was started when the fish attained at least 150 g and ponds were restocked with same quantity of advanced fingerlings (25-50 g) at 15-day intervals keeping the total number of fish same to that of initial stocking. In the first trial, higher production of 3.6 ton/ ha in the high density compared to 2.8 ton/ ha in the low density culture was achieved after 160 days. In the second trial, this model yielded higher production of 3.8 ton/ ha in the high density compared to 3.0 ton/ ha in the low density culture in 180 days. This model with higher density had a BCR of 1.66 suggesting its suitability over the lower density system with BCR of 1.50.

Findings indicated that MSMH model with high stocking density of 15000/ ha can improve production and profitability in low input based milkfish farming. This model could be suitable for small and marginal farmers with several added advantages. The farmers do not require a large capital to meet up various recurring expenditure. After managing the ponds for a maximum 3-4 months, the farmers start earning, which is reinvested for purchase of various inputs required for further fish rearing. Therefore, a marginal farmers can also take up scientific milkfish farming with meagre resources by adopting this system and can meet their

day-to-day needs from fish harvest at regular intervals. Moreover, netting at short intervals results in release of noxious gases and mixing of bottom nutrients with water, which enhances primary productivity of the pond.

6. Culture of long whiskers catfish, *Mystus gulio*

M. gulio is partly marine, euryhaline and suitable for culture in both fresh and brackishwater environments. After the hatchery phase, the yolk-absorbed larvae require to be reared in nursery ponds or cages before stocking to culture pond. This nursery phase is important to obtain better growth and survival in grow-out pond. However, naturally occurring *M. gulio* seeds are widely cultured in the paddy fields and brackishwater areas of West Bengal and Odisha, and in sewage-fed brackishwater system of West Bengal. Moreover, this is a suitable species for polyculture with other fishes.

6.1. Traditional culture

Traditional culture practices depend completely on the natural tidal entry of seed, food and water exchange. Furthermore, traditional systems are often characterized by polyculture with fish or by rotation with rice as practiced in *bheries* of West Bengal and *pokkalis* of Kerala. In this culture system, low lying areas near brackishwater rivers and creeks are encircled by peripheral dyke and tidal water is allowed to enter the impoundment along with natural seeds of various species of shrimps, crabs and fishes. Water is retained with periodical exchanges during lunar cycles and the animals are allowed to grow. After 3–4 months harvesting is initiated partially during lunar cycles. Productivity in this system ranges between 500–750 kg/ ha of which about 30% is constituted by prawns/ shrimps and 70% by fishes including *M. gulio* as one of the species.

6.2. Monoculture

This species can be stocked for monoculture in ponds. In brackishwater *bhery*, it can grow up to 80 g when stocked at 1 no./m^2 in a year. However, high density rearing with a commercial pellet feed (30% crude protein) at the rate of 4 to 6% of estimated biomass daily at densities of 8, 12 and 16 no./ m^2 demonstrated highest productivity of 950 kg/ ha at the highest density. CIBA has developed a monoculture technology of *M. gulio* in brackishwater system. During seven months of farming period at the stocking density of 1 and 2 no./ m^2, it attained an average marketable size of 58.3 g with production of 1000 to 1200 kg/ha. Here, we have used a formulated feed (protein 30% and lipid 6%) as supplementary feed at the rate of 5% of estimated biomass daily. Cost of production came around Rs. 100/kg and the fish

has a ready market of minimum Rs. 250-500/ kg, which is economically lucrative. High density farming (20-40 no./ m^2) in small backyard ponds (300 to 500 m^2) will be an ideal practice.

6.3. Polyculture

M. gulio is a good species compatible in polyculture with other freshwater and brackishwater fishes and shrimp. Polyculture of *M. gulio* at a stocking density of 40 no./m^2 with *Oreochromis niloticus* (60 no./m^2) and *Rhinomugil corsula* (40 no./m^2) gave total production of 3867 kg/ ha than monoculture total production of 1682 kg/ ha in 120 days. *M. gulio* attained an average body weight of 50 g in a culture period of four and half months at the stocking density of 0.1 no./m^2 in polyfarming with *Liza parsia* (stocking density, 0.1 no./m^2) and *Penaeus monodon* (stocking density, 15-17.5 no./m^2).

KRC of ICAR-CIBA took initiatives to standardize polyculture practices with indigenous brackishwater fish and shrimp species, where use of low cost farm made feed with locally available feed ingredients helped the polyculture to be a sustainable and economically rewarding activity. Several experiments using the low-cost feed were conducted in indoor system, on farm and in farmer's ponds to standardize polyculture using indigenous brackishwater fish and shrimp species. Six-species polyculture with different stocking densities, *L. parsia* (5000/ha), *Liza tade* (5000/ha), *Mugil cephalus* (2500/ha), *Scatophagus argus* (2500/ha), *M. gulio* (30000/ha) and *Penaeus monodon* (2500/ha), yielded 4764 kg/ha production using the low cost farm made feed (Rs.25/ kg) having FCR of 1.36, in three consecutive trials of 325 days in farmers pond.

6.4. Rice-cum-fish culture

In monsoon months, along the coastline high rain-fed areas are used for freshwater rice cultivation, which are mono-cropped. After this crop, usually fields remain fallow due to high saline soil, and are used for farming of salt tolerant rice variety and brackishwater fish. Culture of brackishwater prawn and fishes in rice field of West Bengal is an age-old practice. Farming of *M. gulio* and other *Mystus* sp. in rice field is practiced in India and Bangladesh. Total production of fish from rice-fish culture is in range of 500-2000 and 116-605 kg/ha in India and Bangladesh, respectively. Intensification of rice-fish farming was made from low input systems to high input systems. Farming with *Lates calcarifer*, *L. parsia, L. tade* and *M. gulio* at the rate of 10000-15000 no./ ha in rice field production of 1050 kg/ha could be achieved.

6.5. Water quality for *M. gulio* farming

During pond nursery of *M. gulio*, physico-chemical parameters of water should be in the ranges of temperature (25-30^{0}C), salinity (3-5 ppt), transparency (27-40 cm), pH (7.90-8.80), total alkalinity (179-250 ppm), dissolved oxygen (5-8.5 ppm), NO_3-N (1.28-1.36 ppm) and PO_4-P (1.08-1.18 ppm). In *bhery*, physico-chemical parameters of water, such as temperature, salinity, dissolved oxygen, pH, alkalinity, nitrite-nitrogen, total ammonia-nitrogen, nitrate-nitrogen and phosphate-phosphorous range as 14.7-33.6^{0}C, 4.2-19.8 ppt, 5.87-9.58 ppm, 7.85-8.50, 160-168.9 ppm, 0.009-0.024.47 ppm, 0.021-0.044 ppm, 0.069-0.111 ppm and 0.021-0.043 ppm, respectively for *M. gulio* farming. In rice-cum-fish culture of *M. gulio* with other brackishwater fishes and shrimp, water temperature (22.2-32.7^{0}C), total dissolved solids (3.01-5.24 ppm), total ammonia (0.143-0.165 ppm), nitrate (0.091-0.117 ppm), hardness (1589-2500 ppm), transparency (15.2- 22.6 cm), pH (7.1-7.9) and salinity (2.4-15.2 ppt) are optimum.

7. Polyculture of fishes and shrimps

Polyculture is a farming practice where two or more species of fishes are reared together. The concept of polyculture is based on the fact that rearing of two or more compatible aquatic species together will result in higher production compared to monoculture. The underlying goal of polyculture involves increasing productivity by more efficiently utilizing ecological resources within an aquatic environment. Sometimes, one species enhances food availability to other species and thus increases total fish production per unit area. It is commonly believed that polyculture gives higher production than monoculture in extensive and semi-intensive systems and is considered more ecologically sound than monoculture. Before stocking of seeds, pond is prepared well following eradication of pest and predatory fishes, removal of bottom mud and liming, fertilization etc. The ready ponds are stocked with seeds of fish species at 8000-15,000 no./ha along with tiger shrimp seeds of 15,000-30,000 no./ha. The stocking density varies with the quantum of seed availability. Natural pond productivity is maintained by fertilization. In addition, supplementary feed prepared from locally available ingredients can be used at 2-5% body weight daily. This kind of system can yield a total production of 1.5-3.0 tonn/ha in 6-10 months. The preferred species among fishes are: Mullets- *Mugil cephalus* (striped grey mullet), *Liza tade* (tade grey mullet), *L. parsia* (goldspot mullet), Milkfish- *Chanos chanos*, Pearlspot- *Etroplus suratensis* and Tiger shrimp- *Penaeus monodon.*

8. Integrated multi-trophic aquaculture (IMTA) in brackishwater

IMTA is a farming practice which combines cultivation of fed aquaculture species (e.g., finfish/shrimp) with organic extractive aquaculture species (e.g., shellfish/herbivorous fish) and inorganic extractive aquaculture species (e.g., seaweed/ seagrass) in the appropriate proportions to create balanced systems for environmental sustainability, economic stability and social acceptability. The IMTA concept is very flexible and can be land-based (pond/RAS) or open-water systems (cage/pen), brackishwater or marine system. IMTA is well recognized as a mitigation approach against the excess nutrients/ organic matter generated by intensive aquaculture activities especially in brackishwaters, since it incorporates species from different trophic positions or nutritional levels in the same systems. In addition, it is also relevant to implementation of the Ecosystem Approach to Aquaculture (EAA) that is conceptualized and propagated by FAO. Sometimes the more general term 'integrated aquaculture' is used to describe IMTA. The terms 'IMTA' and 'integrated aquaculture' differ primarily in their degree of descriptiveness. Different forms of IMTA are Aquaponics, fractionated aquaculture, integrated agriculture-aquaculture systems, integrated peri-urban aquaculture systems and integrated fisheries-aquaculture systems.

Currently, the existing major IMTA systems in the world are generally simplified with finfish, shellfish and seaweed. The aim is to increase long term sustainability and profitability for the cultivation unit, as the waste of one crop is converted into fertilizer, food and energy for the other crops, which can in turn be sold in market. It reduces adverse impacts on environment while producing economically viable products at the same time. The preferred species for brackishwater IMTA (BIMTA) are: finfishes- seabass, milkfish and mullets; shellfish- green mussel/ oyster; seaweed- *Laminaria* sp., *Gracillaria* sp. or *Kappaphycus* sp. IMTA as viable option to fish farmers in coastal waters in India has not been demonstrated still now. Understanding its potentiality and sustainable nature, all the stakeholders of coastal and marine aquaculture should be encouraged to promote it.

8.1. IMTA trials in brackishwater

The first IMTA model involving mullets (*M. cephalus* and *L. parsia*) and tiger shrimp (*P. monodon*) as fed-species, and estuarine oyster (*Crassostrea cuttackensis*) and seaweed, *Enteromorpha* spp. as extractive species was evaluated as a viable aquaculture option in brackishwater. A 150-day field experiment was conducted in six brackishwater ponds (600 m^2 each). There were two treatment groups, IMTA and polyculture (control) with three

replicate ponds. Ponds under IMTA were stocked with mullets and tiger shrimp at 10000 and 30000 no./ha, respectively, *C. cuttackensis* at 1600 no./ha suspended with basket in water column and *Enteromorpha* spp. at 200 kg biomass/ha. Control ponds were stocked with mullets and shrimp at the same densities to that of IMTA, and devoid of oyster and seaweed. A common low cost polyculture feed was provided to fishes and shrimp. Mullets attained a significantly higher growth in IMTA system compared to that of control ponds, whereas tiger shrimp had insignificantly higher growth in IMTA than in control. A significantly higher production of 1707 kg/ha (19% higher) with better water quality was obtained in IMTA system compared to that of control ponds (1434 kg/ha). There was a significant reduction in apparent feed conversion ratio by 22%, and an increase in net income by 69% and benefit cost ratio by 30% in IMTA system than that of the control. Moreover, for the first time the estuarine oyster, *C. cuttackensis* was used as an extractive species in brackishwater IMTA system. From an indoor trial, it was observed that this oyster species has high water filtration capacity to remove suspended matters including planktons. This preliminary experiment indicates application of IMTA concept in brackishwater as a viable environment-friendly option.

In the second trial, IMTA systems were evaluated in 12 ponds (500 m^2 each) with four different species combinations as treatments: (C) Tiger shrimp (*P. monodon*) + mullets (*M. cephalus* and *L. tade*), (T1) Tiger shrimp + mullets + water spinach (*Ipomoea aquatica*), (T2) Tiger shrimp + mullets + oyster (*C. cuttackensis*), (T3) Tiger shrimp + mullets + water spinach + oyster. Stocking density of different species was kept uniform in different treatments: *M. cephalus*- 2000, *L. tade*- 10000, *P. monodon*- 30000, oyster- 2000 no./ha and water spinach- 300 kg/ha. In this polyculture trial of 150 days, mullets and tiger shrimp served as fed species with oyster and water spinach as extractive species. A significantly higher production (1510 kg/ ha) with better water quality was obtained in T3 system compared to that of other treatments. Additionally, 450 kg vegetable grown on pond dykes was harvested. Economic analysis revealed a significantly higher return (Rs.2.24 lakh/ ha) with BCR (2.14) in T3 followed by that in T1, T2 and C. This trial indicates acceptability of IMTA system with better income and improved environment.

9. Challenges in advancement of brackishwtaer fish culture

The barckishwater aquaculture production potential is not utilized due to several constraints. Few of them are highlighted below.

- Poor infrastructure for farming system in different costal states.
- Unavailability of quality seed as input for finfish culture.
- Improved nursery rearing technology for other finfishes.
- Larval and broodstock diet for commercial hatchery operation.
- Poor health management facilities and disease outbreak.
- Unavailability of uniform leasing policy of land for brackishwater aquaculture in different costal states.
- Poor market intelligence and facilities for marketing of harvested product.
- Poor post-harvest technology.
- Lack of awareness about the new finfishes.

10. Future strategies towards attaining sustainability

The attempt to improve the production from brackishwater aquaculture should focus on following strategies, which will address the above challenges.

- Commercial scale availability of hatchery produced seeds of brackishwater fishes.
- Breed improvement and diversification with finfishes such as seabass, mullets, pearlspot and cobia.
- Establishment of aquatic quarantine and biosecurity system, with suitable capacity building at various levels.
- Availability of quality and affordable feed for farming of different species.
- Comprehensive health management with disease diagnostics and treatment measures for broodfish and larvae.
- Water budgeting and management in aquaculture practices, including treatment and use of wastewater, recycling and multiple use of water and mechanisation in aquaculture.
- Integrated farming systems for enhancing input use efficiency.
- Comprehensive national policy for brackishwater aquaculture.
- Emphasis on infrastructure development for aquafarming site.
- Market information and training on marketing intelligence, with a national data centre for exports of fish products.
- Ensuring food safety through necessary laboratories and trained personnel.
- Rapid detection of pathogens and contaminants in fish products.
- Insurance system for all fishery based commercial activities right from producers to user's level.
- Ensuring financial assistance for fisheries and aquaculture activities from production to consumption level.

11. Conclusion

The aquaculture development project should try to achieve the maximum possible yield, which is not currently possible with existing technology and infrastructure. Development of eco-friendly and cost-effective culture technologies of finfish targeting small-scale farmers is the need of the hour. Some steps towards brackishwater aquaculture development are extension of culture to inland saline areas, bringing more areas under culture, species diversification from existing shrimp to fishes etc. Adequate availability of quality fish seeds will also help in expansion of culture.

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Nursery Rearing Technologies of Brackishwater Fishes

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1. Introduction

Generally, the growth of brackishwater fishes at early life stage is slow. Therefore, after the hatchery phase of 30 days, most of the brackishwater fishes need to undergo a nursery phase where intensive management steps are followed to produce fingerlings in a short span of 6-8 weeks. Through adoption of nursery rearing techniques, fingerlings of stockable size could be produced and stocking of bigger size fish could increase survival and production in grow-out culture. So far, hatchery technologies of four brackishwater food fishes have been developed, such as Asian seabass, grey mullet, milkfish and long whiskers catfish. Nursery rearing being an important and must followed step in brackishwater fish culture, here, we present the details of technologies developed so far for the benefit of readers.

2. Nursery rearing of seabass (*Lates calcarifer*)

2.1. Nursery rearing in hatcheries

Seabass fry of 25-30 days old of 1.0-1.5 cm size can be stocked in 5-10 ton capacity circular or rectangular (RCC or FRP) nursery tanks. Outdoor tanks are preferable. The tanks should be fitted with inlets and outlets. Flow-through provision is desirable. *In situ* biological filter outside the rearing tanks would help in the maintenance of water quality. The water level in the rearing tanks should be 70-80 cm. Tanks should be provided with good aeration facility. After filling with 30-40 cm water and fertilized with ammonium sulphate, urea and superphosphate @ 50, 5 and 5 g (10:1:1 ratio) per 10 ton of water, respectively. The natural algal growth would appear within 2-4 days. In these tanks, freshly hatched *Artemia* nauplii @ 500-1000/L are stocked after leveling the water to 70-80 cm. The nauplii stocked are allowed to grow into biomass feeding with rice bran. When sufficient *Artemia* biomass is seen, seabass fry are stocked @ 800-1000 nos./m^3. The pre-adult *Artemia* would form good food for seabass fry. The fry would not suffer for want of food in the transitional nursery phase in

the tank since the larvae are habituated to feed on *Artemia* in the larval rearing phase. Along with '*Artemia* biomass' available as feed inside the tank supplementary feed mainly minced fish/shrimp meat is passed through a mesh net to make each particle of size of around 3-5 mm and cladoceran like *Moina* sp. can also be given. The fish/shrimp meat feeding has to be done 3-4 times daily. Feeding rate is 100% of the body weight in the first week of rearing. This is gradually reduced to 80, 60, 40 and 20% during 2nd, 3rd, 4th and 5th week, respectively. Regular water change to an extent of 70% is to be done daily. The left-over feed and the metabolites have to be removed daily and aeration should be provided. In a rearing period of 4-5 weeks in the nursery rearing, the seed will be in the size of 1.5 to 3.0 g/ 4-6 cm with survival rate of 60-70%. Adopting this technique at a stocking density @ 1000 no./m^3 in the hatchery, survival rate up to 80% has been achieved. For better survival 'grading' should be done regularly. Vessels/trough placed with different mesh sized nets can be used for grading. When the seeds are left into the containers the seeds will be sieved in different grades according to the mesh size and seed size. Care should be taken that the fry are not injured while handling. If the number is less, it could be manually done.

2.2. Nursery rearing in grow-out site

Rearing fry to stockable size seed in the hatchery itself has some problems. All hatcheries may not have such facilities since the requirement of space will be 5-6 times more than larval rearing space. Maintenance requires additional man power, energy etc. Above all, transportation of large sized seed to culture site would be expensive. To avoid these problems nursery rearing in grow-out site itself can be done wherever possible.

2.2.1. Nursery rearing in ponds

Nursery ponds can be around 200-500 m^2 area with provision to retain at least 70-80 cm water level. Adequate provision for water inlet and water drainage should be provided. Towards drainage side there should be slope. Suitable sized (normally 1 mm) mesh screen nets should be provided in the inlet side and outlet side to avoid entry of unwanted fishes and escape of the stocked fish, respectively. The pond is prepared before stocking. If there are any predator/pest fishes they have to be removed. In case where complete draining is not possible, water level is reduced to the extent possible and treated with Derris root powder @ 20 kg/ha or mohua oil cake @ 2000-3000 kg/ha-m to eradicate unwanted fishes. Use of other inorganic chemicals or pesticides is avoided because these may have residual effects. After checking the pond bottom quality water is filled. If the pond bottom is acidic, neutralization is done

with lime application. In order to make the natural food abundant, the pond is fertilized with chicken manure @ 500 kg/ha keeping the pond water level 40-50 cm. The water level is gradually increased. After 2-3 weeks period when the natural algal food is more, freshly hatched *Artemia* nauplii are introduced. Normally 1 kg of cyst is used for 1 ha pond. These stocked nauplii grow and become biomass in the pond forming food for the seabass fry.

Seabass fry is stocked @ 20-30 no./m^2. Stocking should be done in the early hours of the day. Fry should be acclimatized to the pond condition. Acclimatization for the pond condition is done as follows: the fry in the transport container are emptied into another tank and the pond water is gradually added into the container. This process is continued for a day or two depending upon the difference in the parameters. When the water temperature and salinity in the pond and tank water reach same, fry can be released into the pond. Water is changed @ 30% daily. Supplementary feeding is done with chopped, cooked fish/shrimp meat. The larvae can be weaned to artificial feed at this stage. The feeding rate can be as mentioned earlier. Excessive feeding should be avoided since it would deteriorate the pond condition and also promote filamentous algal growth. The excessive algal growth would deplete dissolved oxygen level in the early hours of the day leading to fish mortality. Hence, excessive algae if any should be removed.

2.2.2. Nursery rearing in net cages (*hapas*)

This method is advantageous to other methods since the management is easier and installation of rearing facility requires less space and capital investment. It can also be extended to any scale depending on the necessity and the capability of the farmer. It can be maintained in one corner of the grow-out pond or near the grow-out cage itself. Since net cages or *hapas* are in *in situ* condition, this will provide conducive environmental condition. The water flow in the cage site would give the fish natural condition. Metabolites and excess uneaten feed will be washed away by the flow of water.

Floating net cages/*hapas* can be in the size of 2×1×1 to 2×2×1 m depending upon necessity. Cages are made with nylon/polyethylene webbings with mesh size of <1 mm. Fry can be stocked @ 400-500 no./m^3. The net cages have to be checked daily for damages; those may be caused by other animals like crabs. The net cages will be clogged by the adherence of suspended and detritus materials and siltation or due to fouler resulting in the restriction of water flow. This would create confinement in the cages and unhealthy conditions. To avoid this, net cages/*hapas* should be cleaned once in a or two-days. Regular grading should be

done to avoid cannibalism and increase the survival rate. Even at higher stocking density of 500 no./m^3 farmer could get survival of 80% in the farm site when the fry were reared in *hapas* adopting the trash fish feeding and other management strategies mentioned above.

3. Nursery rearing of grey mullet (*Mugil cephalus*)

Nursery rearing of stripped grey mullet fry can be conducted in brackishwater tide-fed ponds for production of advanced fingerlings. Among different seed rearing methods, such as only fertilization or feeding, combined fertilization-feeding, fertilization-compost application and fertilization-periphyton systems, the best performances of fish can be obtained in the combined fertilization-feeding and fertilization-periphyton rearing systems.

3.1. Low density fertilization-feeding (FF) system

After treatment of pond bottom with lime, water is taken and fertilized with cattle manure, urea and single super phosphate (SSP) at 500, 30 and 30 kg/ha, respectively. After 7 days of fertilization, ponds are stocked with *M. cephalus* fry (0.55 g/ 36.0 mm) at 15000 no./ha. Formulated feed prepared from locally available ingredients (mustard cake, rice bran, wheat flour, fishmeal etc.) is provided as supplementary feed @ 20 to 5% of body weight. Ponds are fertilized fortnightly with the above mentioned fertilization materials at the same dose. Liming is done at fortnightly intervals with lime stone powder at 250 kg/ha. After 150 days of rearing, grey mullet attains average body weight (ABW) of 96 g.

3.2. High density fertilization-periphyton (FP) system

After bottom treatment followed by water taking according to the method mentioned earlier, ponds are fertilized with mustard cake, urea and SSP at 200, 20 and 20 kg/ha, respectively. After 6 days, bamboo poles are erected vertically in the pond to cover 10% of pond surface area as substrate for periphyton growth. After 10 days of bamboo pole fixing, pond is stocked with *M. cephalus* advanced fry (3.36 g/ 63.7 mm) @ 30000 no./ha. During rearing, all the ponds are fertilized fortnightly with mustard cake at 100 kg/ha. Agricultural lime at 100 kg/ha is applied one day before fertilization throughout the rearing period. Grey mullet fingerlings attain ABW of 28 g in 120 days of rearing.

Table 1: Economics of combined fertilization-feeding (FF) and fertilization-periphyton (FP) systems for grey mullet seed rearing. Calculation is for 1 ha pond and currency mentioned is Indian Rupee.

Operational cost (OC)	**Low density FF**		**High density FP**	
Grey mullet fry	15000 @6/-	90000	30000 @6/-	180000
Feed	2000 kg @35/-	70000	-	
Other inputs		30700		55370
Manpower		12500		10000
Sub-total		203200		245370
Interest on OC @ 10% annually	For 5 months	8467	For 4 months	8179
Total OC		211667		253549
Return from sale of fingerlings				
Grey mullet fingerlings	12630 no. @30/-	378900	28290 no. @20/-	565800
Net return		**167233**		**312251**
Benefit-cost ratio (BCR)		**1.79**		**2.23**

4. Nursery rearing of milkfish (*Chanos chanos*)

Two different systems, such as short duration rearing for fingerling production and long duration rearing for juvenile production can be carried out based on the purpose and facilities available. Fingerlings produced in short time span (45 days) can be transported to other farms, whereas it is difficult to transport juveniles produced in longer time (120 days) to distant farms as it may cause stress and injury leading to mortality to this kind of delicate fish. Therefore, long duration rearing is only suitable when the juveniles are stocked in ponds of the same farm.

4.1. Short duration high density rearing system

After treatment of pond bottom with lime, water is taken to a depth of 30-40 cm and fertilized with mustard cake, urea and SSP at 200, 20 and 20 kg/ha, respectively for the growth of benthic algal complex (lab-lab). After 15-20 days of fertilization, ponds are stocked with milkfish fry (0.2 g/ 30.4 mm) at 100000 no./ha. Formulated powder feed prepared from locally available ingredients (mustard cake, rice bran, wheat flour, fishmeal etc.) is provided as supplementary feed @ 20 to 5% of body weight. Liming is done at fortnightly intervals with lime stone powder at 250 kg/ha. After liming, ponds are fertilized fortnightly with the above mentioned fertilization materials at the same dose. After 45 days of rearing, fingerlings are harvested and attain 8-10 g body weight.

4.2. Long duration low density rearing system

In long duration rearing, milkfish fry are reared in low density either in fertilization-feeding or fertilization-feeding-periphyton system. After bottom treatment followed by water taking according to the method mentioned earlier, ponds are fertilized with mustard cake, urea and SSP at 100, 15 and 15 kg/ha, respectively. After 6 days, white nylon net is fixed vertically in the pond to cover 10% of pond surface area as substrate for periphyton growth. After 15 days of net fixing, pond is stocked with milkfish advanced fry (3.08 g/ 77 mm) @ 25000 no./ha. In both the systems, pellet feed is provided as supplementary feed @ 10 to 2% of body weight. During rearing, all the ponds are fertilized fortnightly with the above mentioned fertilization materials at the same dose. Agricultural lime at 100 kg/ha is applied one day before fertilization throughout the rearing period. Milkfish juveniles attain ABW of 61 g in fertilization- feeding and 76 g in fertilization-feeding-periphyton system in 120 days of rearing.

Table 2: Comparison of economic returns between short duration fertilization-feeding (FF), long duration FF and fertilization-feeding-periphyton (FFP) systems for milkfish seed rearing. Calculation is for 1 ha pond and currency mentioned is Indian Rupee.

	Short duration rearing		**Long duration rearing**		
Operational cost (OC)	**FF system**			**FF system**	**FFP system**
Milkfish fry	100000 @3/-	300000	25000 @5/-	125000	125000
Feed	890 kg @35/-	31150	2800 kg @40/-	112000	112000
Other inputs		14000		28000	30000
Manpower		5000		7500	10000
Sub-total		350150		272500	277000
Interest on OC @ 10% annually	For 2 months	5836	For 4 months	9083	9233
Total OC		355986		281583	286233
Return from sale of fingerlings					
Milkfish fingerlings	85000 nos. @10/-	850000	22000 @20/-	440000	23000 @25/- 575000
Net return		**494014**		**158417**	**288767**
Benefit-cost ratio (BCR)		**2.39**		**1.56**	**2.01**

During harvest, care should be taken by using drag nets made of knotless nylon net or mosquito net to avoid any damage to the fingerlings as they are very delicate in nature. Milkfish fingerling production under combined fertilization-feeding and juvenile production fertilization-feeding-periphyton systems could be a viable option to farmers. Combined fertilization-feeding-periphyton system facilitates to maintain better water quality by

reducing harmful nitrogenous metabolites utilized mainly for the growth of periphytic algal community that serves as natural food to fish. Therefore, this system is environmentally superior to other rearing methods. However, in short duration rearing, fertilization-feeding-periphyton system may provide further higher return.

5. Nursery rearing technology of long whiskers catfish (*Mystus gulio*)

Brackishwater catfish, *Mystus gulio*, also known as long whiskers catfish, is distributed around India to the Malay Archipelago, especially in the estuarine and tidal waters. As a small and indigenous fish species (SIS), it has high market price and increasing demand, and also contains high amount of protein, micronutrients, vitamins and minerals which make it a very desirable candidate for aquaculture in the Southeast Asia. The hatchery produced 15-30 days old fry are further reared in nursery to produce fingerlings suitable for stocking in grow-out system. Nursery rearing in net cages is advantageous to other methods as it is easily managed, and requires less space and capital investment. During the net cage rearing *M. gulio* fry mainly subsists on external feed supply. Among the different feed management practices proven to maximize the benefit of feeding, feeding frequency and ration size play an important role in regulating the feed intake, growth and waste outputs of fish. Therefore, nursery rearing technique considering the right stocking density and feeding frequency results in better output.

5.1. Nursery rearing in ponds

Nursery rearing of yolk-absorbed spawns of *M. gulio* seems to be a critical phase due to switching over from planktonic feeding habit to other feed. Various pond trials showed that 5-7 days old larvae reared at 200-250 no./m^2 can reach 0.5-1.0 g size with survival up to 90% in 30-45 days. Nursery ponds should be fertilized periodically and provided with supplementary feed prepared with finely powdered mustard cake, rice bran and fish meal.

5.2. Nursery rearing net cages

A nursery rearing trial was conducted with 10-day old fry (0.01-0.02 g/ 8-11 mm) in net cages (hapa size: 2×1×1 m) at three stocking densities, 500, 750 and 1000 no./ hapa. Fry were fed twice a day @ 10 to 4% of body weight with a formulated larval diet (CP 30%, CF 6%). After 60 days, fry attained significantly higher growth of 1.31±0.28 and 1.35±0.37 g at 500 and 750 no./hapa densities, respectively compared to that (1.26±0.36 g) of 1000 no./hapa group. However, survival was significantly higher (48.0±1.1%) at the lowest density with

lowest number of shooter emergence (3.8%). Therefore, low density rearing (500 no./ hapa) of *M. gulio* fry is recommended in net cage system.

In another trial, nursery rearing of *M. gulio* fry (0.02 g/ 12 mm) was performed in 12 double-layered net cages (2×1×1 m) with four different feeding frequencies as treatments. Fry were stocked at 300 no./ net cage and fed with a CIBA formulated diet (Crude protein 30%) at 5-15% of the biomass daily for 60 days. Fry attained significantly higher growth of 1.30±0.32 g at 3 times feeding a day compared to other groups. Percentage weight gain and specific growth rate were significantly higher in three times feeding, while there was no significant variation between three and four times feeding. Moreover, higher survival in 3 and 4 times feeding differed significantly from that of 1 and 2 times feeding a day. Therefore, a feeding frequency of 3 times daily is optimum for nursery rearing of *M. gulio* in net cage system

Efficient use of feed with proper feeding frequency helped to minimize feed wastage and shooter emergence. Therefore, it is an important technique in *M. gulio* culture package of practices. The same seed rearing technique followed in farmer's field also proved to be useful and finally resulted in higher production compared to earlier farming methods where no nursery rearing was carried out.

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Cage based spawning and seed production of pearlspot, *Etroplus suratensis* in Recirculatory Aquaculture System: An innovative livelihood model for aqua-farmers

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Pearlspot, *Etroplus suratensis,* also commonly known as green chromide and it is a popular brackishwater food fish in the west coast of India. Owing to its huge demand and the characterstic flavour of this fish caught from the backwaters in south west coast, the Indian state of Kerala has recognized pearlspot as its state fish with the sole objective of conserving the stocks and enhancing the aquaculture production. Pearlspot fetches rates of INR 250 to 500 in markets across the country and it sells for even higher prices in the niche markets. Recently, the fish has also started becoming popular among fish hobbiyists as an ornamental fish. Being a fish with an omnivores feeding habit, aquaculture of pearslpot is considered economical and highly adaptable to different culture systems like pond, pen and cages. A major bottle neck that is limiting the expansion of pearlspot farming is the insufficient availability of quality seed for stocking different growout systems. Although, several studies report the breeding and seed production of pearlspot in earthen ponds, cement tanks and raceways, large scale seed production technology for the species is challenging task due to issues like pair formation, parental care and other factors. To overcome these issues and pave way for a mass scale seed production program, Navsari Gujarat Research Center of CIBA has developed a cage based mass spawning system for pearlspot and the subsequent larval rearing in a recirculatory aquaculture system based hatchery at its research farm in Matwad, Navsari, Gujarat.

Mass spawning of pearlspot in floating net cage

A total of 12 – 15 pairs of pearlspot brooders consisting of both male and female (TL 18 -20 cm & 150 -200 g) can be stocked in floating net cages (4 × 4 ×1.5 m) at a sex ratio of 1:1. The floating net cage can be installed in brackishwater pond for spawning purpose. The

brooders are segregated on the basis of secondary sexual characteristics. Female fish are identified by protruded pinkish enlarged ovipositor whereas, male fish will have whitish pointed genital papilla. Pair formation takes place after 7 – 8 days of stocking in cages.The brooders were fed using formulated pellet diet containing 32 % crude protein and 5 % lipid @ 5% of body weight in two equal feeding ration. Circular clay bowls provided as substrate for breeding purpose. Egg collectors (clay bowls) can be suspended in the cage using nylon twine and tied to the cage collar for easy observation and collection of eggs. The number of egg collectors required would depend on the number of breeding pairs released in to the cage. After providing the substrate, Female lays sticky eggs on the substrate followed by male releases milt on eggs to fertilize the eggs. The number of eggs layed in each spawning depends on the size and condition of brooders. The number of eggs layed in each spawning ranged from 600 to 1250 numbers,with an average fecundity of 900 nos/spawning. The eggs were oblong, heavily yolked, light peach in colour and adhesive in nature. Regular inspection of the egg collection bowls were carried out during evening hrs1800 hours respectively for presence of fertilized eggs. A total 12 – 15 spawnings can be obtained in a month by stocking 15 pairs of brooders in floating cage provided with substrates. The physico-chemical parameters of the pond water for pearlspot spawning in floating net cage :- temperature: 29 – 31^{0}C, salinity: 15 – 25 ppt, Dissolved oxygen: 5 – 6 ppm and pH: 8.1 -8.3.

Collection of fertilized eggs, acclimatization and treatment:-

Fertilized eggs attached to substrate were collected from the cage and the eggs were subjected for hatching. Meanwhile, another substrate was placed in the same position of the cage to facilitate further spawning and to avoid movement of brooders to other location/substrate. Collected eggs with substrate washed repeatedly with clean seawater to remove the debris attached. As a prophylactic treatment, the eggs were subjected for dip treatment with $KMnO_4$ @ 10 ppm for 30 seconds before releasing them into incubation tanks for hatching.

Incubation, hatching and larval rearing in Portable tub based RAS hatchery:-

The fertilized eggs attached to egg collectors were transferred to incubation cum larval rearing tanks attached to a RAS system, A series of 70 liters plastic tubs (LRTs) with inlet and outlet, were placed above a 2 tonne rectangular FRP tank using steel frame installed above the tank. A submersible power head (2500 liter/hr) fitted within the 2 ton FRP tank (reservoir for collection of filtered water) circulated the water between tanks and the

filtration devices (sand and biofliter). Vigorous aeration and mild flow rate of 1 L/min was maintained for incubation and hatching of eggs. The eggs hatched after an incubation period of 2-3 days (48 – 72 hrs) depending on water temperature and egg stage. An average hatching rate of 90 -95 % was observed. After completion of hatching, the substrate was removed and the hatchlings were reared for 20 - 25 days.

The hatchlings of pearlspot measured approximately 5.5 mm in total length and were demersal in nature due to the presence of heavy yolk sac. The newly hatched larvae were stocked in to the plastic tubs (LRTs) at 15 nos./litre. The larvae were fed from the 3rd day onwards using freshly hatched Artemia nauplii at 5 nos./ml. 10th day onwards, larvae were fed twice a day using formulated larval feed (200 µ) and 3 times using freshly hatched Artemia nauplii. After 21 days post hatching, the larvae attained a size of 10-12 mm with a survival rate of 80%. A total of 12000 early fry were produced from 27 spawnings at the hatchery unit within three months using 15 pairs of brooders. The capital cost for setting up one cage unit and a RAS unit for incubation and larval rearing is INR 70,000 that can yield 50,000 fry/annum.

Nursery rearing of pearlspot in hapa based system

Nursery rearing is a very important step in aquaculture for the production of appropriate sized fingerlings. Nursery rearing of pearlspot can be carried out in ponds, tanks and net cages (hapa). However, nursery rearing in hapa is considered superior, as it is economical, easy to monitor and suitable for large scale production of fingerlings in 45 – 60 days of rearing.

Twenty one days old, early fry of pearlspot (0.9 – 1 cm) can be stocked in hapa's (2×1×1 m) installed in earthen ponds @ 500 nos./hapa. Early fry were fed 3 times daily using artificial larval diet at 15 % of the total biomass. The fry attained fingerling size (4 - 4.5 cm), within 60 days of rearing with a mean survival of 80%. During nursery rearing, cleaning of hapas on regular basis is very much essential to avoid clogging and to facilitate water circulation for better growth and survival of the stocked fry.

Advantages of spawning in floating net cages and larval rearing in RAS system:-

- ✓ Pair formation is very easy in cage based breeding model of pearlspot, as it promotes natural selection of pairs within a community of brooders.
- ✓ Risk free maintenance of brooders in cages whereas other systems need expensive RAS system.

- ✓ Enhanced breeding frequency due to complete curtailment of parental care
- ✓ Complete control over egg incubation and larval rearing results in better fry production.
- ✓ Cages can be easily installed in any unutlized water body and establishment of a small RAS system exclusively for larval rearing requires low capital investement
- ✓ Mass scale seed production can be easily achieved from this model.
- ✓ Periphyton attached to the cage mesh forms additional nutrient rich feed for the brooders.

Conclusion:-

Cage based spawning and seed production of pearlspot, *Etroplus suratensis* in a recirculatory aquaculture system can be a promising model for continuous breeding, supply of fertilised eggs and production of 40,000 fry/annum using 15 pairs of brooders. The production of 40,000 fry from a very small setup, is indicative of the ample scope for mass scale seed production of pearlspot. This technology can be propagated to other coastal states of India for mass scale seed production of pearlspot for the benefit of aquafarmers and self-help groups as a livelihood generation activity.

Pearlspot brooder pair

Floating Net Cage installed in pond for pearlspot spawning

Fertilized eggs attached to clay bowl (substrate)

20 day old pearlspot fry

15

Low volume cage culture of brackishwater finfish with special reference to Asian seabass, *Lates calcarifer* and Pearlspot, *Etroplus suratensis*

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1. Introduction

Fish has universally consolidated its position as an affordable health food and increase in fish production ensures income, food and nutritional security. India is bestowed with numerous commercially important, cultivable brackishwater food fishes such as Asian seabass, milkfish, grey mullet, pearlspot, mangrove red snapper, rabbit fish, and estuarine groupers, and ornamentals such as silver moony, spotted scat and orange chromids. Brackishwater sector is largely synonymous with shrimp farming; hence diversification of species and system holds paramount significance to achieve sustainable growth of coastal aquaculture. Recently, due to the availability of hatchery seed and feed, scientific farming of Asian seabass, milkfish, and pearlspot has gained attention from the sector. This needs fine

tuned technology backup for scaling-up the farming in a massive way as a sustainable food production sector. Brackishwater finfish farming is clusters around small stakeholders owning relatively smaller farming areas which necessitate diversification of system in the form of cage, pen etc. to generate alternative livelihood.

India is blessed with plenty of open brackishwater resources such as backwater, creeks, ponds etc. which can be utilized effectively for cage farming of brackishwater finfishes. Each coastal states have huge scope for brackishwater aquaculture since every state

is bestowed with vast resources of brackishwater areas including the estuaries, mangrove based creeks, canals, lagoons and backwaters. Presently, the percentage of area utilized for brackishwater aquaculture is very low and the figures emphasize the untapped potential of brackishwater aquaculture to bring about a significant difference in fish production and livelihood generation in different coastal states of India.

Hence utilization of these water bodies for low volume cage farming will be a model for brackishwater finfish production and livelihood generation coastal rgions. Low volume brackishwater cage aquaculture is a powerful tool to utilize the untapped water resources for enhancing fish production, productivity and translating these into income for the stakeholders involved. Production of high value food fish in cages is one of the most promising aquaculture based livelihood options in these water bodies. It enables utilization of open water bodies for fish production and livelihood generation. It will also enable skill development in cage construction, cage setting and carrying out different phases of farming such as nursery, pre grow-out and grow-out using underutilized open waters. Among candidate brackishwater aquaculture finfish species, Asian seabass Lates calcarifer and pearlspot Etroplus suratensisis are high value food fish with high growth rates and suitable for cage culture in brackishwater bodies.

2. Low volume low cost seabass and pearlspot cage culture- an overview

CIBA has developed a comprehensive low volume low cost cage culture technology for sustainable and viable farming of seabass and pearlspot, which can be adopted by farmers. The most important factors which contribute to the success of cage culture of fish species are the availability of stock size seed, cost effective feed, cage management and active participation of the farmers. These factors have direct impact on production and sustainability of culture system. Cages are fabricated using galvanized iron, PVC or bamboo for constructing the frames to support knotted or knotless cage nets; these are floated with barrels in brackishwater creeks as well as mangrove waters. However, low volume cage culture is a low venture setup which can be adopted in backyard creek and water bodies. The cage culture rearing phase is of 180-240 days depending upon the culture practice. Production of high value fish using low volume cages in brackishwater bodies can thus be a potential livelihood option for the poor and the low volume cages even can be fabricated by the farmers themselves. This type of cage culture is becoming increasingly popular amongst the small

fish farmers, Self Help Groups, tribal communities as livelihood options in different coastal states, Kerala, Karnataka, Maharashtra, Andhra Pradesh and Tamil Nadu.

Cage culture of seabass and pearlspot in brackishwater creeks of Palghar, Maharashtra

Cage culture of seabass and pearlspot in brackishwater creeks of Sindhudurg, Maharashtra

Cage culture of seabass and pearlspot in brackishwater creeks of Sindhudurg, Maharashtra

3. Species for cage culture

3.1 Asian seabass

Asian seabass (*Lates Calcarifer*) known as Bhetki or barramundi in India is one of the commercially important finfish species caught from inshore areas, estuaries, backwaters, lagoons and freshwater areas. Seabass is a fast growing species with ability to tolerate wide fluctuations in environmental conditions and gaining rapid popularity as a candidate species for diversification in coastal aquaculture in India. Seabass is carnivorous in nature. However, juveniles are omnivores. Seabass is an opportunistic predator, whose diet pattern changes at different ages as per growth. It feeds mainly on zooplankton (rotifers and artemia nauplii) during larval phases and as they grow, starts feeding on small aquatic vertebrates and invertebrates (juvenile fishes and shrimps). They show a preference for pelagic fish rather than benthic crustaceans as the prey is large. However, juvenile seabass even consumes smaller sizes of seabass of the same age group and can cause reduction in the survival rate due to cannibalism. It is one of the fastest-growing fish, can grow to an average size of 1.0-1.2 kg in 8-10 months and fetches good price in domestic and international market. It is considered as a potential candidate species for farming in saline or freshwater environments in ponds and cages.

Asian seabass, *Lates calcarifer*

3.2 Pearlspot

Pearlspot *Etroplus suratensis* (Bloch, 1790) is commonly called as green chromide and is popularly called as Karimeen (Malayalam) and Kalunder (Marathi). It is the state fish of Kerala having high market demand in the west coast. Pearlspot is an economically important food fish having a market value of Rs. 250-500 per kg depending upon the size. It is also an emerging ornamental fish. Pearlspot is adaptable to different culture systems like ponds, pens and cages. Being omnivorous in nature, aquaculture of pearlspot is economical and highly suitable for supporting livelihood of small-scale fish-farmers.

Pearlspot, *Etroplus suratensis*

4. Site selection for the cage farming

Site selection is a critical factor determining the success of the cage culture operation. Cage culture sites must be free of any kind of water pollution and located away from local sewage discharges. The site should have regular water depth of above 3 m and must retain at least 2.5 m of water level during lowest low tide of the year. Wind and wave action should be at a moderate level (< 0.1 m/s) and must have good water exchange through cages to replenish dissolved oxygen and remove waste metabolites. The selected sites should be such that cages can be easily monitored and managed during culture operation. The site should not be a regular fishing ground or boat navigation channel. The site should also be free from any sand mining area as removal of sand from water led to suspension of minute soil particles, inorganic and organic sediments in water column cause clogging of nets, reduces water exchange and dissolved oxygen levels. Preferably, the site should be near to shore with boat connections and good approach road for easy access.

Pollution free creek

5. Cage structure and fabrication

5.1 Cage frame

The cage mainframe and whole structure can be made of galvanized iron (GI) pipes, PVC pipes or bamboo poles as per the requirement. However, cage frame should be strong and of good quality. The GI pipes painted with anti-rusted epoxy paint have good shelf life in brackishwater bodies. To fabricate single grow-out cage with the dimension of 4 m x 4 m x 2 m (32 m^3) about twelve numbers of 1.5 inch and six numbers of 1.25 inch GI pipes are required. The cage frames and structures need to be coated with epoxy paint to avoid the GI pipes rust in saltwater. To maintain the whole cage net in vertical position, square shape internal frames of 2 m x 2 m size can be made of 1.5 inch GI Pipes and kept inside at bottom of each cage net compartment. For construction of grow-out net, cages frame of 4 m x 4 m size are used to support the cage nets.

GI pipes cutting and wielding for fabrication of cage frames

Wielded cage frames

Wielded cage frame

5.2 Floatation and anchoring devices

Floatation and anchoring devices are used for maintaining the position of the cage in the water bodies. The whole structure of the low volume cage is kept afloat with the help of six HDPE barrels of each 210-250 L capacity. Mild stainless steel structures (85 - 100 kg) or concrete weight blocks can be used as anchors in creeks having a muddy or sandy bottom. Anchors have five legs to withstand the strong water current and avoid cage structure displacement during tidal fluctuations. The four corners of internal frame are tied with PP rope for quick uplifting of the frame during cage cleaning, changing net, etc.

Cage frame installation

Anchoring cage frames

5.3 Cage nets

Two types of cage nets can be used for pre-grow out and grow out seabass culture to achieve good survival and higher production from the system. In the pre-grow-out cage culture (around 90 days culture period), seabass fingerlings (1-5 inch & 2-15 g) are stocked in the cage. In this case, HDPE knotless cage nets of 4m x 4m x 2m dimension are made of 24/36 ply & 12-20 mm diamond/ hexagonal shape mesh webbing mounted with 3-6 mm rope of 2000-3000 kg tensile strength. In the grow-out culture cages, advanced fingerlings in the size of 50-100 g seabass can be stocked. For this purpose, HDPE knotless cage nets of 4m x 4m x 2m dimension made of 45/60 ply and 25-30 mm diamond/hexagonal shape mesh webbing mounted with 6-12 mm rope and 3000-5000 kg tensile strength are suitable till end of the culture period.

Asian seabass is carnivorous fish in nature. Hence, to avoid cannibalism and improve survival during pre-grow-out cage culture, net cages are provided with two internal partitions (each 2 m x 2 m x 2 m). This would facilitate the restocking of two different size group seabass fishes in each compartment. In the case of pearlspot culture, such internal partition is not required as they are herbivore fishes. All sides of the pre-grow out and grow-out cage nets are supported with nylon strap of 3 mm width and 1 mm thickness with inbuilt 6 mm and 12 mm polypropylene ropes for tying the nets to the cage mainframe. To protect the farmed fishes from wild predatory (crab and other fishes) organisms, each cage should have an outer

protection cage net in the dimension of 5 m x 5 m x 2.5 m made of HDPE net of 40 mm mesh size.

Fixing cage nets to cage frame

Partitioned net cages for seabass pre-grow out cage culture in creeks

6. Management practices followed during low volume cage culture

6.1 Stocking of fish

Fish stocking density and duration of the culture period are the two important aspects that need to be decided well in advance before initiating the culture activity. Stocking should

be done in the early morning hours in between 07:00-11:00 to avoid stress to the fishes. Healthy fish seeds free of injury and disease should be used to stock the cages. In the pre-grow-out cages, seabass fingerlings with the size of 3 to 5 inch can be stocked at 20-30 nos./m^3 i.e. 640-960 nos./cage, whereas pearlspot seed with the size of 1 - 2 inch size can be stocked @ 90-100 nos./m^3, i.e. 2880-3200 nos./cage. The density can be increased depending upon the creek's water quality and proper feed management should be done. However, higher stocking densities of seed over and above optimal level can lead to stress and infections due to crowding and management problems. Hence optimal stocking density should be maintained in both pre-grow-out and grow-out culture systems.

Stocked seabass and pearlspot fingerlings

Transportation of fish seed for stocking in cages

Seed stocking in cages

6.2 Feed and feeding practices

Supply of quality feed of appropriate size with essential nutritional value is of utmost importance to yield better productivity. Fishes stocked in the cages can be fed either with extruded/floating or slow sinking pellet feeds. Generally, supply of trash fishes to feed seabass can be avoided. Procurement of the live or dead trash fishes on a regular basis is challenging and these fishes also poses threats of pathogens, if not processed properly. However, formulated feed has relative advantages over trash fishes with respect to procurement, storage and feeding.

Seabass fish prefers slow sinking pellet feed (2-6 mm) having protein 40-45% and FCR for pellet feed is claimed to be around 1.5 to 1.8 depending upon the feeding strategies followed by the farmer.

Pearlspot is herbivorous fish, which feed mainly on filamentous algae, aquatic macro-vegetation and planktonic organisms. Pearlspot also readily accept artificial feed and the FCR is about 1.5 to 1.8. Pearlspot prefers floating feed (0.6-2 mm) with protein requirement of 25-35%. Feeding is done twice a day (morning and evening). The fish should be fed at the rate of 10% of their body weight to start with, further the feed requirement is calculated based on the biomass. Feeding rate vary from 3-10% of total biomass over a period. After four to six weeks, the feeding rate may be reduced to 8%. As the fish grow in size the feeding rate should be gradually reduced to 5%, 3% and even 2% finally. The total biomass in the cage culture should be fortnightly estimated by random sampling of 15-20 fishes from each cage for adjusting the feed ration. Overfeeding needs to be avoided, leading to poor water quality of the culture system and resulting in reduced survival rates.

Floating and sinking feeds for seabass

Farmer feeding formulated feed to seabass and pearlspot stocked in cage

6.3 Size grading to reduce cannibalism and optimize survival rates

Seabass is highly cannibalistic fish and if proper timely feeding, clearing and management care is not taken then it affects the survival as well as sustainability of the culture. Hence, sizes wise grading of farmed fishes is one of the important steps during pre-grow out cage culture of seabass. This is very much essential to reduces the cannibalism and improve the percentage survival during cage culture. Pearlspot are not carnivorous species hence fish grading is not required in case of pearlspot cage culture. However, cages need to be checked for chocking with debris if any and clearing purpose. Fingerlings size grading

need to be done at 15-20 days intervals. During the grading, all fingerlings are removed from each cage nets and taken in grading containers where shooters are separated from the smaller size fingerlings group and kept separately according to their sizes. The smaller ones kept in one compartment and larger ones (shooter) are segregated into other cage compartment. Grading must be done in early morning (07:00-11:00 am) and late evening (16:00-18:00 pm) hours. At least, 3-4 people are essentially required on each cage for proper grading, cage net cleaning, cage management and to avoid handling stress to cultured fishes.

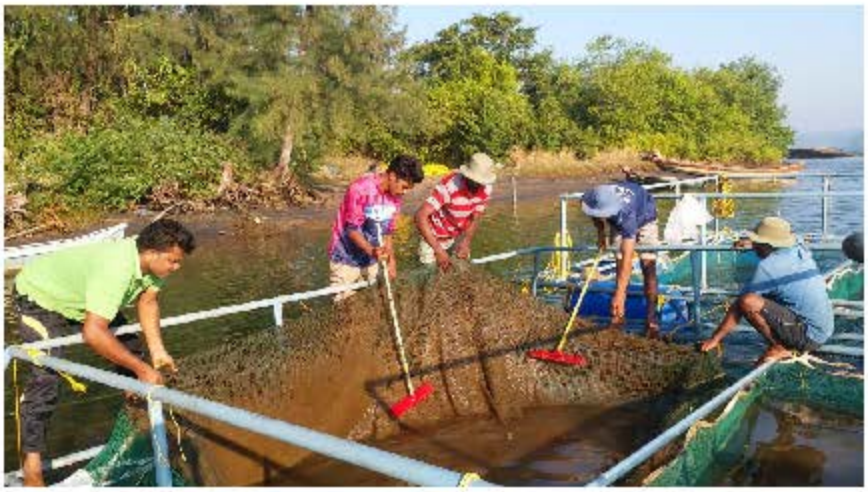
On site seabass grading by farmers

Cage culture graded shooter seabass

Cage culture graded small seabass

6.4 Routine cleaning and maintenance of cages

Since cages are inside the water and exposed to water current, debris materials tend to accumulate over the period. Cage mesh clogging with mud and debris will reduce water exchange lead to accumulation of waste products, deplete the oxygen and causes stress to fishes, affecting feeding and overall growth. The fouling organism will also attach and clog the meshes. Other animals like crab may damage the nets. The cages should be regularly checked for clogs and leaks. Any damaged nets should be repaired or replaced. The cages need to be cleaned regularly with brushes to avoid clogging of the cage nets.



Cage net cleaning by farmers

6.5 Monitoring the water quality

Water quality is a crucial aspect of the cage culture. The selected site should be free of any water pollution to avoid stress on the cultured species. Sites with turbid water must be avoided as they clog net cages and affect fish growth most of the time. Site should have

following water parameter quality; dissolved oxygen: > 5ppm, temperature: 25-33°C, pH: 7.5-8.5, water current: 0.1-0.2 m/s, salinity: 5-35 ppt, ammonia: < 00.25 ppm, Nitrite: <0.05 ppm, Nitrate: <1.00 ppm, turbidity: 10-30 NTU's.

7.0 Harvesting and marketing:

Seabass and pearlspot attain a marketable size of 800-1,000 g and 200-250 g respectively, within culture period of 6-8 months depending upon culture conditions and management. The best harvesting size for seabass is above 1.0 kg as it fetches a higher price in the market due to consumer preference of this size. Prior to harvesting, feeding is stopped and harvesting needs to be done either in morning or evening hours to maintain the freshness and quality of the harvested fish. After harvesting, fishes need to be immediately iced with flake ice @ 1:1 ratio and then transported to the market in the basket. Regular partial harvest and supply of fish in monsoon season even can give more profit to the farmers. The market price of 0.5-1.0 kg seabass can range from Rs. 400-600/kg while 150-200 g of pearlspot is about Rs. 250-300/kg.

Fish harvesting from cages by farmers

Harvested seabass from cages

Harvested pearlspot from cages

8.0 Conclusion

The coastal fisher communities required an alternative livelihood and income generation option. Brackishwater areas like creeks, lagoons, estuaries etc. can be well utilized for taking up for low volume cage culture rearing of different candidate brackishwater finfishes. Cage

culture rearing technologies for diverse species maybe adopted up by coastal fisher communities as a very effectively a sustainable enterprise for their livelihood.

Details of the material required for battery of 4 cages: 1 unit

Sl. No.	Items	1 Cage	04 Cages
1	GI pipes -1.5 inch	12	48
2	GI pipes -1.25 inch	06	24
3	1.5 Inch elbows for two cage frames	08	32
4	Pre grow out cage net of 10-12 mm mesh	01	04
5	Grow out cage net of 30mm mesh	01	04
6	Outer cage net of 40 mm mesh	01	04
7	200 L air tight barrels for floating cage	06	24
8	250 L feed barrels	02	08
9	50 Kg anchors	02	08
10	Grading tanks	02	08
11	Pigeon net	01	04
12	Hand net	02	08
13	3 mm rope bundle	01	04
14	Painting brush	02	08
Common items for 4 cages-1 unit			
16	pH meter	01	
17	Dissolved oxygen kit	01	
20	Refractometer	01	
21	Thermometer	01	
22	Ammonia kit	01	
23	Nitrite kit	01	
24	Nitrate kit	01	
25	Turbidity kit	01	
26	Weighing balance	01	
27	6 mm Rope bundle	01	
28	12 mm Rope bundle	01	
29	Epoxy paint	08 litre	
30	Tarpaulin sheet	03	
31	12 feet cement poles for cage land-based false shed	08	

1. Estimated capital cost and operational cost required for rearing of Asian seabass and pearlspot in 4 cages-1 unit

Sl. No.	Item	For 4 cages (Rs. In lakhs)
A Capital cost		
1	GI pipes (1.5 inch - 48 nos.; 1.25 inch - 24 nos.)	01.30
2	HDPE knotless 4m x 4m x 2m pre-growout cage net of 20 mm mesh - 04 nos. HDPE knotless 4m x 4m x 2m growout cage net of 30 mm mesh - 04 nos. HDPE knotless 5m x 5m x 2.5m outer cage net of 40 mm mesh - 04 nos.	01.20
3	Cage anchors (50 kg) - 2 nos. & floating airtight barrels (210 L) - 24 nos.	00.75
4	Big feed storage IBC tanks (1000 L) - 02 nos.	00.10
5	Fish grading tanks: 08 nos.	00.25
6	Water proof digital weighing balance - 01 (For weighing fish and feed)	00.10
7	Water parameter meters & kits - 01 each (refractometer, thermometer, ph meter, dissolved oxygen, alkalinity, hardness, ammonia, nitrite, nitrate, turbidity)	00.20
8	One False Shed/4 cages (for feed, cage material storage) Silpaulin sheet for false shed (18 x 24 feet) - 01 nos. Silpaulin sheet for false shed (12 x 18 feet) - 03 nos. Cement Poles -04 nos.	00.10
9	G.I Pipe Elbows - 48 nos.	00.05
10	Life jackets - 08 nos.	00.15
Total (A)		**04.20**
B Operational cost		
1	Cost of 3000 seabass fingerlings (5-6 inch; 40-50g) @ Rs. 80/fingerling (Density 1000 nos./cage).	02.40
2	Cost of 3000 pearlspot fingerlings (3-4 inch; 8-10g) @ Rs.16/fingerling (Density 3000 nos./cage).	00.48
3	Seabass feed (3-6 mm)-900 kg/cage @ Rs. 110/kg (expected FCR 1:1.5)	02.97
4	Pearlspot feed (0.8-1.8 mm)-600 kg/cage @ Rs. 70/kg (expected FCR 1:1.5)	00.42
5	Feed, material & local transportation	00.10
6	Miscellaneous expenses (wielding charges, antifouling paint, ropes, boat charges big fish hand nets, etc)	00.43
Total (B)		**06.80**
C	**Grant total (A+B)**	**12.00**

2. Expected output: Farmers employment and income through cage culture/year

Sl. No.	Particulars	Seabass culture	Pearlspot culture
1	Number of cages (nos.)	03	01
2	Farmers per unit (nos.)	08	
3	Culture duration (days)	240-300	240-280
4	Density/cage (nos.)	1000	3000
5	Fingerlings stocking (nos.)	3000	3000
6	Seed survival (%)/cycle	70	90
7	Expected size of fish at harvest (g)	1000	200
8	Expected production (kg)	2100	540
9	Fish selling price (Rs./kg)	500	300
10	Revenue from sale of seabass & pearlspot fish (Rs. in lakhs)	10.50	01.62
11	Total revenue from 4 cages (Rs. in lakhs)	12.12	

3. Economics & revenue details

Sl. No.	Particulars	Amount (Rs. In Lakhs)
A	Capital cost*	00.84
B	Operational cost	06.80
C	Total expenditure	07.64
D	12% interest on total expenditure	00.92
E	Total cost/year (A+B)	08.56
F	Revenue from sale of fish /year	12.12
G	Net profit / Year (E-F)	03.56
H	Net profit / 08 farmer	00.45

****Capital cost is used for five years***

Brackishwater aquaculture for food employment and prosperity

16

Importance of Live Food Organisms in Brackishwater Fish Larval Rearing

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Introduction:

One of the major bottlenecks in the hatchery production of finfish is the larval rearing which includes the transition from an endogenous to an exogenous feeding by the larvae. There are two types of finfish larvae: *precoicial* and *altricial*. In *precoicial* larvae, when yolk sac is exhausted, they look as mini adults, having fully developed fins and mature digestive system including functional stomach. These fishes can ingest and digest formulated feeds as a first feed. *eg*: salmon and trout. In case of *altricial* larvae, when yolk sac is exhausted (3-4 days in most of the tropical fishes), they remain in relatively undeveloped state. The digestive system is rudimentary, lacking a stomach and much of the digestion takes place in the hind gut epithelial cells. Such a digestive system seems to be incapable of processing formulated diets. Moreover, the larvae are inefficient to catch and chase their food due to underdeveloped vision and other sensory functions.Fishes at the time of their first feeding are quite fragile and delicate creatures. It is the most *critical phase* of their life when they need right type of nourishment for their survival and growth. If this requirement is not met, they perish. So it's necessary to give live feeds at this stage.

Mainly live feeds consist of phytoplankton and zooplankton grazed upon by economically important fishes.They include different group of organisms like, microalgae, rotifers, artemia and copepods. Importance of live feed is due to several factors as small size, rich essential nutrients, broad spectrum composition of food, better intake due to the movement, auto-digestion characteristics, facilitate better nutrient assimilation in larvae, stimulate feeding behaviour due to soft texture and attractability and ample scope for enrichment. This is why live feed organisms are called *living capsules of nutrition*. Adequate quantity of these live feed should be present during larval rearing phase for the successful larval rearing. Hence, the mass cultures of these live feeds are required to meet the need of the hatchery operation.

MICRO-ALGAE

Introduction

Phytoplankton comprises the base of the food chain in the marine environment. Therefore, micro-algae are indispensable in the commercial rearing of various species of marine animals as a food source for all growth stages of bivalve molluscs, larval stages of some crustacean species, and very early growth stages of some fish species. Algae are furthermore used to produce mass quantities of zooplankton (rotifers, copepods, brine shrimp) which serve in turn as food for larval and early-juvenile stages of crustaceans and fish. Besides, for rearing marine fish larvae according to the "green water technique" algae are used directly in the larval tanks, where they are believed to play a role in stabilizing the water quality, nutrition of the larvae, and microbial control. All algal species are not equally successful in supporting the growth and survival of a particular filter-feeding animal. Suitable algal species have been selected on the basis of their mass-culture potential, cell size, digestibility, and overall food value for the feeding animal. Various techniques have been developed to grow these food species on a large scale, ranging from less controlled extensive to monospecific intensive cultures. However, the controlled production of micro-algae is a complex and expensive procedure. A possible alternative to on-site algal culture is the collection of algae from the natural environment where, under certain conditions, they may be extremely abundant. Furthermore, in order to overcome or reduce the problems and limitations associated with algal cultures.

Major classes and genera of cultured algal species

Today, more than 40 different species of micro-algae, isolated in different parts of the world, are cultured as pure strains in intensive systems. The list includes species of diatoms, flagellated and chlorococcalean green algae, and filamentous blue-green algae, ranging in size from a few micrometer to more than 100 µm. The most frequently used species in commercial mariculture operations are the diatoms Skeletonema costatum, Thalassiosira pseudonana, Chaetoceros gracilis, C. calcitrans, the flagellates Isochrysis galbana , Tetraselmis suecica , Monochrysis lutheri and the chlorococcalean Chlorella spp.

➢ Culture techniques

Microalgae can be produced using a wide variety of methods, ranging from closely-controlled laboratory methods to less predictable methods in outdoor tanks. Various chemical media are used for indoor and outdoor cultivation. (Guillard's F/2

medium, Walne's medium, Johnson's mediumetc). There are five different stages in the growth of every microalga (Fig 1). The best quality microalgae can be harvested/utilized during the late exponential growth phase.

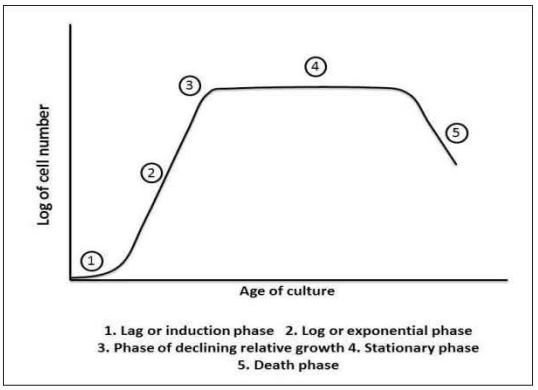

Fig: 1. Different phases in life cycle of microalgae

Indoor culture allows control over illumination (3000-500 lux), temperature (24 ± 1^0C), nutrient level, contamination with predators and competing algae, whereas outdoor algal systems make it difficult to grow specific algal cultures for extended periods. Open cultures such as uncovered ponds and tanks (indoors or outdoors) are more readily contaminated than closed culture vessels such as tubes, flasks, carboys, bags, etc. Axenic cultures are free of any foreign organisms such as bacteria and require a strict sterilization of all glassware, culture media and vessels to avoid contamination. Even then, using strict management measures and continuous monitoring it is possible produce mass cultures of microalgae in open outdoor tanks / ponds for hatchery operations. Different types of microalgae cultures are described in the following section.

✓ **Batch culture**:

The batch culture consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density. In practice, algae are transferred to larger culture volumes prior to reaching the stationary phase and the larger culture volumes are then brought to a maximum density and harvested. The following consecutive stages might be utilized: test tubes, 2 liter flasks, 5 and 20 liter carboys, 160 liter cylinders, 500 liter indoor tanks, 5,000 liter to 25,000 liter outdoor tanks (Fig 2).

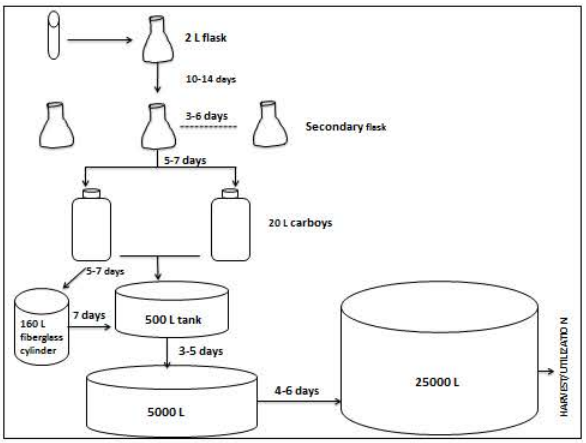

Fig: 2. Diagrammatic representation of batch culture of microalgae
(Source: FAO fisheries technical paper: 361)

✓ **Continuous culture**

The continuous culture method, i.e. a culture in which a supply of fertilized seawater is continuously pumped into a growth chamber and the excess culture is simultaneously washed out, permits the maintenance of cultures very close to the maximum growth rate. Two categories of continuous cultures can be distinguished: *Turbidostat* culture, in which the algal concentration is kept at a preset level by diluting the culture with fresh medium by means of an automatic system. *Chemostat* culture, in which a flow of fresh medium is introduced into the culture at a steady, predetermined rate. The latter adds a limiting vital nutrient (e.g. nitrate) at a fixed rate and in this way the growth rate and not the cell density is kept constant.

✓ **Semi-continuous culture**

The semi-continuous technique prolongs the use of large tank cultures by partial periodic harvesting followed immediately by topping up to the original volume and supplementing with nutrients to achieve the original level of enrichment. Semi-continuous cultures may be indoors or outdoors, but usually their duration is unpredictable. Competitors, predators and/or contaminants and metabolites eventually build up, rendering the culture unsuitable for further use. Since the culture is not harvested completely, the semi-continuous method yields more algae than the batch method for a given tank size. Large outdoor ponds either with a natural bottom or lined with cement, polyethylene or PVC sheets have been used successfully for algal production. The nutrient medium for outdoor cultures is based on that used indoors, but agricultural-grade fertilizers are used instead of laboratory-grade reagents.

✓ **Microalgae production chain in finfish hatchery**

A finfish hatchery must be equipped with a well maintained indoor microalgae laboratory to keep pure cultures different strains used. The temperature, light intensity and aeration/CO2 supply should be controlled inside the lab. Sterilized media are used in the lab.

The culture volume ranges from 10 ml to 20 litres in the lab. The inoculum for intermediate culture is provided from the indoor laboratory. Generally, 20 litre carboy cultures are used as inoculum for intermediate culture. The intermediate culture contains 500-1000 litre FRP tanks in a room with transparent roof and adequate ventilation. The cultures from intermediate room are used in large outdoor tanks (5-20 tons).

Commonly used microalgae species in fish hatchery are: *Chlorella salina*, *Chlorella vulgaris*, *Nannochloropsis occulata*, *Isochrysis galbana*, *Tetraselmis* sp, *Thalassiosira pseudonana* etc.

❖ **Nutritional perspective of microalgae**

The nutritional value of any algal species depends on its cell size, digestibility, production of toxic compounds, and biochemical composition. Although there are marked differences in the compositions of the micro-algal species, protein is always the major organic constituent, followed usually by lipid and then by carbohydrate. Expressed as percentage of dry weight, the range for the level of protein, lipid, and carbohydrate are 12-35%, 7.2-23%, and 4.6-23%, respectively. The content of highly unsaturated fatty acids (HUFA), in particular eicosapentaenoic acid (20:5n-3, EPA), arachidonic acid (20:4n-6, ARA), and docosahexaenoic acid (22:6n-3, DHA), is of major importance in the evaluation of the nutritional composition of an algal species to be used as food for marine organisms. *Isochrysis galbana* is rich in DHA, whereas *Nannochloropsis occulata* is rich in EPA.

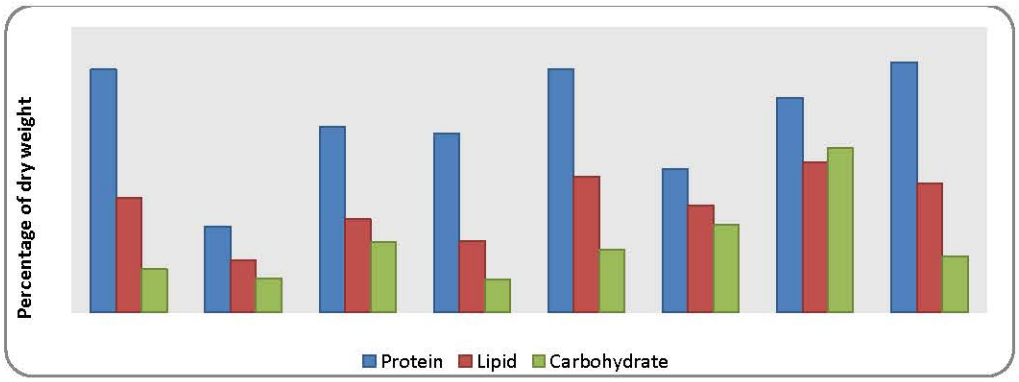

Fig 3. Concentrations of protein, lipid and carbohydrate in some species of micro-algae commonly used in aquaculture

✓ **Commonly used media for indoor microalgae culture**

WALNE'S MEDIUM FOR ALGAL CULTURES

Recommended for large volumes of aquaculture strains

Stocks		**per 100 ml**
(1)	Trace metal solution (TMS)	
	$ZnCl_2$	2.1 g
	$CoCl_2.6H_2O$	2.0 g
	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.9 g
	$CuSO_4.5H_2O$	2.0 g
	Make up to 100 ml with distilled water. This solution is normally cloudy. Acidify with a few drops of conc. HCl to give a clear solution.	
(2)	Vitamin solution	
	Vitamin B_{12}. (Cyanocobalamin)	10.0 mg
	Vitamin B_1 (Thiamine.HCl)	10.0 mg
	Vitamin H (Biotin)	200.0 µg
	Make up to 100 ml with distilled water.	
		per litre
(3)	Nutrient solution	
	$FeCl_3.6H_2O$	1.3 g
	$MnCl_2.4H_2O$	0.36 g
	H_3BO_3	33.6 g
	EDTA(Disodium salt)	45.0 g
	$NaH_2PO_4.2H_2O$	20.0 g
	$NaNO_3$	100.0 g
	TMS (1 above)	1.0 ml
	Make up to 1 litre with distilled water.	

Medium	**per litre**
Nutrient solution (3)	1.0 ml
Vitamin solution (2)	0.1 ml
Sterilised seawater	1.0 litre

Dispense nutrient and vitamin solutions separately into 10 ml and 1 ml respectively and autoclave at 15 psi for 15 minutes. Add an aliquot of each aseptically to 10 litres of sterilised seawater.

f/2 Medium

Stocks		**per litre**
(1)	$NaNO_3$	75g
(2)	$NaH_2PO_4.2H_2O$	5.65g
(3)	Trace elements (chelated)	
	Na_2EDTA	4.16 g
	$FeCl_3.6H_2O$	3.15 g
	$CuSO_4.5H_2O$	0.01 g
	$ZnSO_4.7H_2O$	0.022 g
	$CoCl_2.6H_2O$	0.01 g
	$MnCl_2.4H_2O$	0.18 g
	$Na_2MoO_4.2H_2O$	0.006 g
(4)	Vitamin mix	
	Cyanocobalamin (Vitamin B_{12})	0.0005 g
	Thiamine HCl (Vitamin B_1)	0.1 g
	Biotin	0.0005 g

Medium	**per litre**
$NaNO_3$	1.0 ml
$NaH_2PO_4.2H_2O$	1.0 ml
Trace elements stock solution (1)	1.0 ml
Vitamin mix stock solution (2)	1.0 ml

* Add while stirring

Make up to 1 litre with filtered natural seawater. Adjust pH to 8.0 with 1M NaOH or HCl. For agar add 15g per litre Bacteriological Agar. Sterilise by autoclaving for 15 minutes at 15 psi and use when cooled to room temperature.

✓ **Fertilizer used for outdoor microalgae culture**

(Ammonium sulphate: Urea: Super phosphate 10:1:1; grams per 100 litres of seawater)

❖ **Rotifers as live feeds**

Introduction

Although Brachionus plicatilis was first identified as a pest in the pond culture of eels in the fifties and sixties, Japanese researchers soon realized that this rotifer could be used as a suitable live food organism for the early larval stages of marine fish. The successful use of rotifers in the commercial hatchery operations of the red sea bream (Pagrus major) encouraged investigations in the development of mass culture techniques of rotifers. Twenty five years after the first use of rotifers in larviculture feeding several culture techniques for the intensive production of rotifers are being applied worldwide. The availability of large quantities of this live food source has contributed to the successful hatchery production of more than 60 marine finfish species and 18 species of crustaceans. To our knowledge, wild populations of rotifers are only harvested in one region in the P.R. China, (i.e. the Bohai Bay saltworks) where Brachionus plicatilis is used as food in local shrimp and crab hatcheries.

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 (0.7-1.4 off- spring.female-1.day-1). Moreoever, 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, resides in the possibility of rearing these animals at very high densities. Even at high densities, the animals reproduce rapidly and can thus contribute to the build- up of large quantities of live food in a very short period of time. Last, but not least, the filter-feeding nature of the rotifers facilitiates the inclusion into their body tissues of specific nutrients essential for the larval predators (i.e. through bioencapsulation; see further).

Types of rotifers:

Generally, three types of rotifers are cultured around the world in finfish commercial hatcheries depending on the size requirements:

1. SS (Super Small) type: 100 – 140 µm
2. S (small) type: 141 – 220 µm
3. L (large) type : >220 µm

- **Specific requirements to start the mass culture:**

For starting rotifer mass culture, algal cell density should be $>10\times10^6$ cells/ml for *Nannochloropsis* sp or *Chlorella* sp. Algae culture should be 2 -5 times higher than the volume of rotifer culture. Temperature should be in the range of 27 – 28° C. FRP tanks/ Concrete cement tanks are suitable to start the culture.

- **Pure culture of rotifer:**

Rotifers are easily available in coastal areas where the water is abundant with nutrients. To start a pure culture, 50 – 60 liter water can be sieved through 50 – 80 µ mesh size net. This filtered water contains different species and strains of rotifers. Preferred species can be selected and isolated individually under microscope. These isolated single rotifers can be put in culture tubes with algae water for further reproduction under diffused light. After every 12 hour fresh algae should be supplied to maintain the algal cell density. Gradually increase the volume to 25 ml, in 50 ml beakers. Change the culture daily once. Use 50 - 80 µ mesh to separate the rotifers. Continue this procedure, till the density reaches 50 individual / ml and the volume up to 500 ml. Increase algal cells density to 3-4 million cells/ml. When the density exceeds the above, remove half of the quantity and mix clean sea water to make up the quantity.

- **Mass culture of rotifer**

Start the microalgae (*Chlorella*/ *Nannochloropsis*) culture in the rotifer culture tanks and when the culture reaches the density of 20×10^6 cells/ml, inoculate pure culture of rotifer to achieve an initial density of 10 individuals/ml. Allow the culture for 7-8 days to increase the rotifer density. Harvest and concentrate the rotifers using 50 µ mesh plankton net. After each harvest, thoroughly clean the tanks with fresh water. Culture of live feed should be scheduled to ensure daily harvest for uninterrupted production. Better reproduction and nutritional quality can be achieved by regulating feed, water, temperature, salinity and aeration during the culture process.

- **Rotifer enrichment**

The nutritional quality of rotifers depends on their food source. Highly unsaturated fatty acids (HUFA) are essential for the survival and growth of the larvae. Rotifer feeds containing DHA and EPA can be valuable for marine and brackishwater fish larvae. Depending upon their food source, rotifers are composed of about 52-59% protein, up to 13% fat and 3.1% n3 HUFA. 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. The harvested / concentrated rotifers can be kept in these microalgae cultures for few hours for enrichment. The ratio of EPA:DHA can be manipulated by using different proportions of algae for enrichment. There are plenty of commercial enrichment media are available in the market for rotifers.

❖ **Artemia as live feed**

Among the live diets used in the larviculture of finfish, nauplii of the brine shrimp Artemia constitute the most widely used food item. Annually, over 2000 metric tons of dry Artemia cysts are marketed worldwide for on-site hatching into 0.4 mm nauplii. Indeed, the unique property of the small branchiopod crustacean Artemia to form dormant embryos, so called *'cysts'*, may account to a great extent to the designation of a convenient, suitable, or excellent larval food source that it has been credited with. Those cysts are available year round in large quantities along the shorelines of hypersaline lakes, coastal lagoons and solar saltpans scattered over the five continents. After harvesting and processing, cysts are made available in cans as storable 'on demand' live feed. Upon 24 hour incubation in seawater, these cysts release free-swimming nauplii that can directly be fed as a nutritious live food source to the larvae of a variety of aquatic organisms, which makes them the most convenient, least labour-intensive live food available for aquaculture. Approximately 90 % of the world's commercial harvest of brine shrimp cyst comes from the Great Salt Lake in Utah. All the life stages of Artemia, the decapsulated cyst, nauplii, juvenile, and sub adults are used as feed.

High densities of hatching from cysts can be achieved with transparent funnel shaped containers (20 -30 liter, cylindro-conical FRP tanks) that are aerated from the bottom, which keeps all cysts in suspension condition. Illumination in hatching tanks is provided by 60 watt fluorescent lamp from a distance of 20 cm. Complete hatching takes place within 24 -36 hours. After complete hatching, nauplii can be collected by attracting them near light source.

Salinity	30 - 35 ppt
pH	7.5 – 8.5
Temperature	27 – 30° C
Oxygen	>2 ml/l
Illumination	>1000 lux
Cyst density	1 gm/liter

Table 1: Conditions required for artemia cyst hatching

The hard shell and chorion can be removed by a technique, decapsulation, to achieve higher hatching percentage. Here, the cysts are hydrated in water for 30 minute and then dipped in sodium hypochlorite (NaOCl) solution of 200 ppm for a while. During this time care should be taken so that temperature should not exceed beyond 30° C. When cyst becomes orange in colour, aeration should be stopped and cysts are washed with Sodium thiosulphate solution for less than a minute to remove the residual chlorine. Cysts are to be rinsed with water and can be kept for incubation in hatching tank with strong aeration in filtered seawater with minimum illumination of 2000 lux for 18-24 hours. After complete hatching, the aeration can be stopped and all other light sources are to be turned off and a light should be provided near bottom outlet for nauplii collection. After 10-15 minutes, nauplii can be collected with the help of fine mesh net.

❖ **Copepods as live feed**

Most of the commercial finfish species are reared using rotifers and Artemia nauplii since they can be cultured in large quantities at high densities. Unfortunately, using rotifers and Artemia during this early period in life history does not always promote optimal larval growth since these live preys may contain an inadequate fatty acid profile and, in some cases, be of an inappropriate size. Obtaining enough copepods of desired stages at a specific time has been one of the barriers for extensive use in aquaculture and experimental work with fish larvae or other copepod-feeding organisms. Therefore, establishment of reliable production methods for copepods that can meet the quantitative requirements of larval fishes is essential.

Copepods are nutritionally superior to all other live feeds commonly used in marine and Brackishwater aquaculture. This is the one of the main reasons by which copepod culture has got immense importance in finfish larval nutrition. A number of beneficial effects have been linked to copepod nutrient composition in relation to early larval nutrition. In particular, emphasis has been put on lipid composition, and the content and ratio of the polyunsaturated fatty acids (PUFA) docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA)

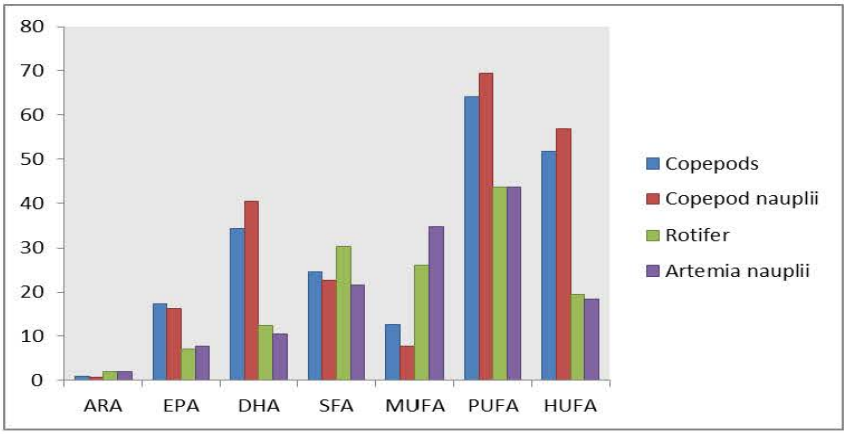

Fig 4. Fatty acid composition of various live feeds (Source: Meeren *et al.*, 2008)

In contrast to rotifers, copepods are more difficult to culture on a commercial basis. Only a few species of copepods have been mass cultured successfully. Many temperate copepods produce resting eggs as a common life-cycle strategy to survive adverse environmental conditions, which is analogous to *Artemia* and *Brachionus* sp. These characteristics can be made useful in aquaculture.

❖ Conclusion

Since the live feed are inevitable for larval rearing of many species of finfish, the research on new vistas in live feed nutrition is the need of the hour. Apart from commonly used live feeds like microalgae, artemia and rotifers there are many more live feed organisms with high nutritional profile are yet to explore. The availability of on-grown live food would not only offer farmers and exporters a better alternative option for feeding their fish, but more importantly, the possibility of enhancing the fish performance and quality through bio-encapsulation. There are several potential live feed organisms which are to be addressed for mass culture techniques. The researches in the area of live feed nutrition will enhance the successful and effective larval rearing of many marine and brackishwater finfishes.

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17

Common fish diseases and its management in brackishwater farm and hatchery

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Being one of the fastest growing sectors of India, brackishwater aquaculture is a very important sector both in terms of meeting rural livelihood and export potential. However at present days, the brackishwater aquaculture production is very much hampered due to spreading of different diseases. During 2017-18, India earned ` 45,107 crores through export of fish and fishery products. Although, brackishwater aquaculture sector is mostly dominated by different species of shrimp, there are many economically important finfish species including Asian seabass (*Lates calcarifer*), goldspot mullet (*Liza parsia*), grey mullet (*Mugil cephalus*), tade mullet (*Liza tade*), milkfish (*Chanos chanos*), nona tengra (*Mystus gulio*), fourfinger threadfin (*Eleutheronema tetradactylum*), etc. The occurrence of diseases in brackishwater aquaculture system can be controlled to some extent by proper management practices, which includes proper biosecurity measures, maintenance of good water quality, judicious use of aquaculture medicines, etc.

Diseases of brackishwater finfishes

Viral nervous necrosis (VNN)

Viral nervous necrosis (VNN) is caused by β-nodavirus. This disease affects different marine and brackishwater fish species causing a very high mortality, especially in larval and juvenile stages. The alternative name of this disease is Viral Encephalopathy and Retinopathy (VER). In India, the disease was first reported in Chennai in 2005 and later on, it was reported in other parts of country. A wide range of fish species including *Lates calcarifer* (Bhetki), *Mugil cephalus*, *Chanos chanos* (milkfish), *Epinephelus tauvina*, *Sardinella longiceps*, *Amblygaster clupeoides*, *Mystus gulio*, *Leiognathus splendens,* etc are affected by this virus. The disease also affects freshwater fish like Nile tilapia (*Oreochromis niloticus*) and the ornamental fish guppy (*Poecilia reticulata*). The transmission of this disease take place both via vertical and horizontal route. This disease is usually transmitted through influent contaminated water, introduction of infected juvenile fish, implements and

translocation of fish from one location to another etc. Very often, different species of fish acts as carriers of this virus. The affected fishes exhibit dark colouration of the body, loss of appetite and spiral swimming. The mortality rate is higher in juveniles. The diagnosis can be done either by histopathology or by RT-PCR. Till date, no effective treatment method or commercial vaccines are available for this disease.

Diseases caused by Iridoviruses

Iridovirus infection usually affects Asian seabass (Bhetki or *L. calcarifer*). This disease is caused mainly by two groups of DNA viruses, *Ranavirus* and *Lymphocystivirus*. The viruses coming under genus Ranavirus causes systemic disease leading to heavy mortality and economic loss, while *Lymphocystivirus* is known to cause localized infection. The affected fish becomes anaemic and lethargic. The mortality rate of *Ranavirus* infection depends upon different factors like water temperature, water quality, age and other culture environment and it varies greatly from 0 to almost 100%. Spleen and kidney tissue are the most desirable organ for detection of this virus. The detection of this virus is mostly based on different immunological techniques e.g. IFAT (Indirect Fluorescent Antibody Test), Abnormally enlarged cells with very deep stain are observed under histopathological examination of liver and spleen with Giemsa stain. The molecular detection of this pathogen can also be done by PCR. Maintenance of good water quality, stocking of pathogen free fish and avoidance of overcrowding and overfeeding etc can help in prevention of this disease. For red sea bream Iridovirus infection, a formalin-killed vaccine is commercially available.

Diseases caused by *Vibrio* spp.

Vibriosis is caused by infection by different species of *Vibrio* including *V. anguillarum*, *V. salmonicida*, *V. harvei*, *V. alginolyticus, V. parahaemolyticus*, *V. vulnificus,* etc. Vibriosis in finfish takes place throughout the year. However, the incidence is more in summer season, Common symptoms of vibriosis include anorexia, lethargy, darkening of body, and reddened ulceration of body haemorrhage at mandible, isthmus, bases and rays of fins. The ulceration with haemorrhage is observed on different parts of body surfaces and due to this symptom, this disease is also known as 'Ulcerative Haemorrhagic Septicaemia'. The enlargement of spleen (splenomegaly) is very often observed. Although it affects fish of all age group, the disease is more severe at nursery stages. The diagnosis of the disease is generally done by isolation of particular *Vibrio* spp. and different immunological methods (e.g. slide agglutination test using specific antisera). The suitable target organ for diagnosis is

brain and kidney. Vaccination of seabass against *V. anguillarum* serotype O1 with a degree of success has been reported. Aquavac *Vibrio* Oral®, which is meant for vaccination of trout against vibriosis, has also been successfully used for prevention of vibriosis in Asian seabass.

Infection by *Aeromonas* spp.

The bacteria under genus *Aeromonas* usually cause opportunistic infection. This group of organisms is generally normal inhabitant of both brackishwater and freshwater aquatic system. Normally, these bacteria do not cause disease condition in fishes. But when the cultured species are under stressed condition, these opportunistic pathogen attacks fish causing diseases. These stressed conditions include sudden fluctuation of pH, high temperature, depletion of dissolved oxygen, increase in the level of different toxic gases (H_2S, nitrite, ammonia and CO_2), etc. In case of brackishwater fishes, *Aeromonas* infection is mostly caused by *A. hydrophila, A. caviae* and *A. punctate.* This disease is characterized by haemorrhage on different parts of body, mostly near fin and tail with varying degree of mortality. In severe cases, erosion of tail and fin is also observed. The isolation of the organism from the affected body parts can be considered as confirmatory diagnosis of this disease. Starch-ampicillin agar and Rimler-Shotts agar containing novobiocin are considered as suitable selective media for isolation of *Aeromonas* spp.

Epizootic Ulcerative Syndrome (EUS)

This disease is also known as 'Red spot disease' because of presence of red ulceration throughout the body of the affected fish. The causative agent is an oomyceteous fungus, *Aphanomyces invadans*. However, very often EUS is also associated with secondary bacterial infection. This disease is a very common among wide varieties of freshwater fishes causing heavy economic losses to the fish farmers. Among brackishwater finfishes, the disease often affects *Mystus gulio* (Nuna tengra), different species of mullets (*Mugil* spp., *Liza* spp.) and Asian seabass. Milkfish are generally resistant to EUS. The spread of diseases generally takes place through water-borne transmission of zoospores, contact between fishes and introduction of infected fish into non-infected ponds. On the head of affected fishes, haemorrhagic ulcers are frequently observed and it very often extends to skull leading to exposure of brain. EUS in freshwater fishes is more prevalent mainly in two seasons i.e. at the beginning of winter and during rainy season generally after heavy rain. However, the mortality rate is higher during winter season. The disease is diagnosed by visual symptoms and ulcerative lesions.

The confirmation can be done by microscopic observation non-septate branchial hyphae at the periphery of the lesions.

Columnaris disease

The etiology of the disease is a bacterial pathogen named *Flexibacter columnaris*. This disease is mostly important in different freshwater fishes. However among brackishwater fishes, Asian seabass especially at juvenile and nursery stages are susceptible. This disease is characterized by saddle-shaped lesion in the mid-body position near dorsal fin and is mostly associated with over-stocking, poor hygiene and skin trauma. The affected fish can be treated by the dip treatment with copper sulfate (2 ppm for 2 minutes) or with potassium permanganate (5 ppm for 15-30 minutes).

Velvet disease

This disease is very often seen in Asian seabass. *Amyloodinium ocellatum*, a dinoflagellate is responsible for this disease. The organism attaches to gill filaments or body surfaces of the affected fish. This causes necrosis of gill and skin and is evidenced by dark discolouration of the body. High stocking density and high level of organic matter are predisposing factors of infection with dinoflagelletes. This disease is often associated with observation of white spots on the gill. Very high mortality may be observed if not treated in time. Diagnosis can be be done by microscopic observation of gill and skin of affected fish The affected fish can be dipped in 200 ppm of formalin for 1 h or in 0.5 ppm copper sulfate for 5 days.

Diseases caused by ciliates group of protozoa

The ciliated protozoan infection in fish is mostly caused two species of ciliated protozoa *Cryptocaryon* spp. and *Trichodina* spp. *Cryptocaryon irritans* is generally found on gill and external surface of the fish. The *Cryptocaryon* infection in fish is also known as 'White spot disease' due to presence of numerous white spots on the body surfaces. The excess mucus is also produced from the affected body part. Over-crowding and low water temperature are generally considered as predisposing factors for infection with *Cryptocaryon*. *Trichodina* spp. is another important ciliated protozoa, which causes trichodiniasis in Asian seabass. This protozoa attaches to skin and gills. The affected fish exhibit severe respiratory distress due to heavy mucus production around the gills. The disease can be controlled by formalin or acriflavin bath.

Crustacean infection

The important crustacean parasites of the brackishwater fishes are *Caligus* spp. (sea lice), *Ergasilus* spp. (gill maggots) and *Lernaea* spp. (anchor worm) are important. All of them affect Asian seabass. These parasites are usually transmitted through water, live feed, wild fish and contaminated tools and equipment. Affected fishes show anorexia with sluggish behavior. Erosion is observed in gill and skin leading to secondary bacterial infection. High mortality generally takes place, if not treated in time. The introduction of wild fish in the pond may aid in spread of these infection in the culture system. Heavy infestation with copepods results in mechanical damage, impaired respiration, petecheal haemorrhage, anaemia and emaciation. These parasites also act as mechanical vectors for other bacterial and viral pathogens. The diagnosis can be done by microscopic examination of skin and gill.

Flukes infections in brackishwater finfishes

The common skin fluke of fishes are *Benedinea* spp. and *Dactylogyurs* spp. They generally affect body surface, eyes and occasionally gills. The commonly affecting gill flukes of fishes are *Gyrodactylus* spp. and *Diplectanum* spp. Poor water quality such as low pH, high stocking, high nitrates and nitrite levels are the predisposing factors. The most common symptom is respiratory distress. The fishes often come to surface and preferably near aeration equipment for getting more oxygen. Areas of haemorrhage with ulcers, which is very often circular in shape, are generally observed. In severe infection, the body of the fish is covered by a slime layer. The affected fishes become prone to secondary bacterial infections.

Argulosis or Fish Louse

This disease is caused by an ectoparasite, *Argulus*, which is also popularly known as fish lice. Morphologically, these parasites are dorsoventrally flattened. They are commonly found in the skin and fins of freshwater fishes and to a lesser extent in brackish water fishes. The affected fish exhibit haemorrhagic ulcers, which is produced by the trauma due to attachment of this parasite. Very often, these haemorrhagic ulcers are associated with secondary bacterial infection. Affected fish show loss of appetite, lethargy and irritation. It is practically difficult to eradicate *Argulus* in culture waters as the adults and larval stages are active swimmers. Infested fish can be treated with formalin or organophosphorus insecticides. Drying the ponds and tanks between cycles will reduce *Argulus* infestation.

Some common treatment measures of Brackishwater fin shrimp and finfish diseases:

It is always advisable to administer aquaculture medicine only after proper consultation with aquaculture experts or fish health professional. Haphazard and indiscriminate use of any chemical or medicine must be avoided. Indiscriminate use of medicines not only affects the health of cultured aquatic species adversely, but also increases the risk of export rejection if banned chemicals or medicines are in use.

However, the common treatment methods are depicted below:

Treatment for Epizootic ulcerative syndrome (EUS) in fin fish:

Quick lime can be applied @ 15 kg per 1000 m^2. After 1-2 days of application of lime, potassium permanganate can be applied @ 1 kg per 1000 m^2.

Copper sulfate: Copper sulfate can be applied in the pond. However, the dose is very crucial. The maximum dose depends upon the alkalinity of the pond water. The maximum dose of copper sulfate is X/10 kg per hectare, where X stands for alkalinity of pond water in ppm. Otherwise, the affected fish can be dipped in copper sulfate solution (4 mg/Litre) for 5 minutes for consecutive 3 days.

Application of CIFAX @ 1 Lit per hectare area. For heavy infection, the dose may be repeated after 7 days.

For bacterial infection in finfish:

Application of potassium permanganate: Potassium permanganate may be applied at the rate of 20 kg per hectare in pond. In case of fin fish, the dip treatment of the affected fish in potassium permanganate solution can be done. The affected fish may be separated and dipped in potassium permanganate solution (20-50 mg/Liit) for 10-30 minutes. This dip treatment can be repeated for 3 days.

Application of lime: Application of quick lime can be done at the rate of 8-10 kg per 1000 square meters. Lime application may be followed by application of potassium permanganate (1 kg per 1000 m^2). Very often it is recommended apply quick lime and potassium permanganate mixed with sand. This is done especially when the pond bottom is adversely affected.

Benzalkonium chloride: For control of pathogenic bacteria, benzalkonium chloride (50%) can be applied @ 5 Lit per hectare.

Application of suitable probiotics: Probiotics are the live beneficial bacteria, which can reduce the harmful and pathogenic bacterial load. For control of harmful bacteria in pond water, different water probiotics and soil probiotics may be applied. On the other hand, for controlling pathogenic bacterial infection in shrimp or finfish, gut probiotics can be given along with feed. Different commercial probiotics marketed by different aquaculture companies are available. Manufacturer's instruction should be strictly followed before application.

Treatment of parasitic disease:

Application of formalin: The parasitic infection in fish can be controlled by application of formalin in pond water @ 15-20 Liters per hectare. The dip treatment with formalin can also be done in 150-200 ppm formalin for 5 minutes under sufficient aeration.

Acetic acid treatment: The affected fish may be dipped in 1000 ppm glacial acetic acid for 1-2 minutes.

Dichlorovos: This may be added to pond @ 20 kg per hectare area.

Health management in brackishwater finfish at hatchery level:

Health management in hatchery level is extremely important both for quality and quantity of seed production. Prevention is considered as the first step in controlling infectious diseases in hatcheries. Simple measures like sanitizing hands before handling fish, having foot dips with disinfectants at all entry points in the hatchery, disinfecting the source water etc. can help a lot in controlling infections. Some of the important aspects in hatchery level health management are as follows:

1. Quarantine: All brooders brought to the hatchery need to be quarantined till it is tested and found to be free of any diseases or parasites. The location of the quarantine tank should be away from the actual hatchery site. Brooders brought to the hatchery must be inspected for any diseased condition and they also need proper acclimatization with the quarantine tank.
2. Biosecurity: Strict biosecurity measures need to be adopted to prevent the possible entry of pathogens into the hatchery. The following measures need to be adopted in a fish hatchery.
    a. Ideally the hatchery should be located away from general public and the entry to the hatchery should be restricted to authorised personnel only.
    b. Visitors to the hatchery should follow strict sanitary guidelines.
    c. Vehicles entering the hatchery should pass through a disinfectant pit.

d. The source water should be filtered properly to prevent the entry of unwanted pathogens. The water also should be disinfected properly with UV treatment or ozonization.

e. Each building /culture area must be provided with foot dips containing potassium permanganate.

f. The hatchery personnel need to sanitize their hands before any hatchery operation.

g. Entry of bird, rodents or any other stay animals must be prevented.

3. All the hatchery tools including hand nest, beakers, siphon tubes, etc. should be disinfected periodically (at least once in a day).
4. The entire hatchery premises should be disinfected between two seasons.
5. All the hatchery tanks must be disinfected between two batches of production.
6. The live feed samples used for hatchery may be tested periodically to know the presence of pathogens.
7. Aeration to the tanks should be stopped briefly in the morning allowing debris and dead larvae to settle down. The bottom debris may be siphoned out.
8. Different water quality parameters like dissolve oxygen, ammonia, nitrate, nitrite, temperature etc. should be checked daily. Optimum water quality should be maintained.
9. Excess feeding of the larvae should be avoided as it will deteriorate the water quality resulting in reduced survival.
10. In case of high mortality, the cause of the mortality should be identified at the earliest. Larval and water samples should be sent to laboratory for testing. Further the source of the infection needs to be ascertained to prevent further infections. For this all input materials like feed and water should be tested.
11. Use of probiotics in larval rearing water is reported to be beneficial and is said to increase survivability and reduce mortality. Hence suitable probiotics may be used at the recommended dose.
12. The total bacterial load and *Vibrio* load in the inlet water should be monitored regularly. If any increase in the load is observed, suitable measures should be taken to disinfect the water.
13. Higher stocking densities of larvae should be avoided as it leads to stress and bacterial infections.
14. All dead fish and larvae must to be disinfected before disposal.
15. Aseptic techniques must be followed for collection of samples from hatchery. The catheters, needles etc. used in sampling should be properly disinfected before collection of samples.
16. The broodfish must be handled carefully to avoid any stress and injury. To minimize stress different anaesthetics like phenoxy ethanol, clove oil, etc. may be used before

sampling. Mullets broodstocks are very sensitive to handling and a special care must be taken for handling the broodstock of this species.

17. The periodical inspection of brooders must be done for presence of parasites.
18. Vaccinate the brooders for diseases which are endemic, if vaccines are available.
19. The hatchery personnel must be educated properly for all the biosecurity and sanitary measures.

Conclusion:

The occurrence of different diseases and resultant production losses are considered as the major limiting factor for the rapid growth of the brackishwater aquaculture. For any aquaculture diseases, the prevention is always better than cure. In this perspective, it is worthy to mention that the two key aspects of disease management of aquaculture is following a good culture practice and adoption of strict biosecurity measures both at farm and hatchery level. Formulation of effective policy and strict implementation of guidelines by regulatory authorities may also play a crucial role in minimizing the risk of diseases leading to increase in brackishwater aquaculture production.

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Development of fish vaccine and its application to control viral diseases

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Introduction

World fish production reached 179 million tonnes in the year 2018 out of which aquaculture has contributed 82 million tonnes [1]. Aquaculture production has peaked in the recent years due to intensification of aquaculture and species diversification. The high growth rate in aquaculture also had a price to pay. Intensive culture practices has resulted in several disease outbreaks resulting in production loss. Among the diseases, viral diseases are of significance since they spread rapidly causing acute mortalities. They are not easily amenable to any treatment measures. Disease management includes establishing good biosecurity protocols, adopting good management practices, early diagnosis of disease and treatment. Since most viral diseases do not have a specific antiviral drug which can control and treat the infection, prophylaxis is the only reliable method of controlling viral diseases.

Viral diseases of cultured fish

Viruses cause 22.6% of the infections in cultured finfishes. However control of the viral infections is difficult due to the rapid spread of the infection and unavailability of specific chemotherapeutants. The viral infections of the farmed finfish along with the causative agent, clinical signs, hosts affected are summarised in the table 1.

Table 1: Viral disease of farmed finfish

Disease	Host species	Pathogen	Viral morphology	Viral genome
Koi herpesvirus disease	carps and carp varieties such as Koi carp and ghost carp	Cyprinid herpesvirus 3	icosahedral symmetry, size: 170–230 nm	dsDNA, 277 kb
Infectious haematopoietic necrosis	Salmons and trout	Infectious haematopoietic necrosis virus	Enveloped, bullet shaped 160 x 90 nm	non-segmented, negative-sense, single-stranded RNA
Epizootic haematopoietic necrosis	Redfin perch and Rainbow trout	Epizootic haematopoietic necrosis virus	large (150–180 nm) icosahedral virus	ds stranded linear DNA genome 150-170 kb

Infectious Pancreatic Necrosis	Rainbow trout, brook trout, Brown trout, Atlantic salmon	Infectious pancreatic necrosis virus	Non-enveloped, icosahedral measuring about 60nm in diameter	bi-segmented dsRNA virus
Lymphocystis Disease	Herrings, Smelts, batfishes, killifishes, scorpion fishes, sea basses, sunfishes, etc.	Lymphocystis disease virus-1	icosahedral virus, approximately 200-300 nm in diameter	single linear double stranded DNA of 102.6 kbp
Oncorhynchus masou Virus Disease	Salmon and Rainbow trout	salmonid herpesvirus type 2 (SalHV-2)	200-240 nm, enveloped, icosahedral	dsDNA
Spring viraemia of carp	Common carp, grass carp, silver carp, bighead carp, crucian carp, goldfish etc.	Spring viremia of carp virus	bullet-shaped, 80–180 nm in length and 60–90 nm in diameter	negative sense single stranded linear RNA of ~11 kb
Viral hemorrhagic septicemia	Rainbow trout, turbot, Japanese flounder as well as a broad range of wild freshwater and marine species	Viral hemorrhagic septicemia virus	bullet shaped measuring about 180 nm long and 60 nm in diameter	12-kb negative single-stranded RNA
Viral encephalopathy and retinopathy	Asian sea bass, , European sea bass, turbot , halibut, Japanese parrotfish, red-spotted grouper, and striped jack	Nervous Necrosis Virus	icosahedral, non-enveloped, 25-30 *nm* in diameter	two positive-sense RNA molecules-RNA1 (3.1kb) and RNA2 (1.4 kb)
Carp pox	carp and koi carp	Cyprinid herpesvirus-1	icosahedral , Enveloped	dsDNA, 291 kb
Red sea bream iridoviral disease	red sea bream and more than 30 other species of cultured marine fish	red sea bream iridovirus	Non-enveloped, icosahedral, 200–240 nm in diameter	dsDNA, 112 kb

Vaccines

A vaccine consists of a killed or avirulent or attenuated pathogen as a whole or a part of it which can stimulate the immune system of an animal to produce a specific response and memory. Upon a natural infection the already primed immune system of the animal mounts a

prompt response thereby producing specific antibodies against the pathogen resulting in the elimination of the pathogen from the host.

Properties of viral vaccines

Following are the ideal properties of a viral vaccine

- The vaccine should be safe to the host, the vaccinator and the consumer without causing adverse reactions or vaccine marks in the host.
- The vaccine should produce long lasting immunity ideally till the production cycle of the fish.
- The vaccine should be able to induce cell mediated, humoral and mucosal immunity.
- The vaccine should be 100% effective and should be effective against all strains and serotypes of the viral pathogen in a wide variety of hosts.
- The virus present in the vaccine should not regain virulence.
- The vaccine should be easily administrable.
- The vaccine should be economical.
- The vaccine should have a long shelf-life.
- The vaccine should not pose ethical issues in licencing.

Immune response to vaccination

The innate immunity comprising of molecules such as interferon are induced in quick response to vaccination. Subsequently, the adaptive immune system of fish comes into play when the fish encounters a pathogen or when immunized. Immunoglobulin M (IgM) is the major immunoglobulin of fish. The B lymphocytes upon antigen presentation secrete IgM and are found in the serum and mucous of gill, skin and intestines. The secretion of IgM is maximum in case of intraperitoneal vaccination. IgM is also secreted into the mucus when immunised by immersion or by oral routes. In addition to mucosal IgM, systemic IgM is also produced upon immersion and oral vaccination. IgT is reported to be an intestinal immunoglobulin equivalent of that of IgA in mammals and is produced in the intestines upon exposure mucosa associated lymphoid tissue [2]. IgD is also a mucosal immunoglobulin, the transcripts of which are upregulated many fold in immersion vaccinated fish suggesting that this immunoglobulin plays a major role in mucosal immunity [3].

Types of viral vaccines

There are several types of viral vaccines and new types are being developed continuously. The vaccine types commonly available are as follows.

Killed vaccine

Killed vaccines consist of virulent pathogen which has been inactivated by chemicals or by heat. The virus no longer can multiply in the host. They are easy to produce and are economical. The virus is usually inactivated by heat or formalin or by a combination of both.

Live attenuated vaccine

Live vaccines consist of pathogen which has been rendered incapable of causing an infection or contain an avirulent strain of the pathogen. The virus is attenuated by passaging it in an unnatural host for prolonged time till its infectivity is lost. Live vaccines have the advantages that they can stimulate both cell mediated and humoral immunity. Further the vaccine virus can multiply in the host and results in longer immunity.

Subunit vaccine

Subunit vaccine consists of a portion of the viral pathogen viz. a particular protein or a peptide which can induce an immune response in the host against the pathogen.

Recombinant vector vaccines

Recombinant vector vaccines consist of an avirulent virus having antigenic components of a virulent virus. Thus the live virus cannot produce an infection while the virulent virus portion present in the recombinant virus can mount an immune response. The vaccine has both the advantages of live vaccine and a subunit vaccine.

DNA vaccine

A DNA vaccine consists of a plasmid vector containing a portion of the viral genome which codes for some of the immunogenic proteins of the virus. Once administered, the DNA vaccine synthesizes the viral protein using the host cell machinery, thus expressing the viral protein in the host which results in an immune response against the pathogen.

Autogenous vaccine

An autogenous vaccine is usually a killed vaccine prepared using the pathogen isolated form the epizootic for which a licenced vaccine is not available. Autogenous vaccines are prepared to reduce the loss due to diseases till licensed vaccines are made available.

Vaccination methods

The route of vaccine administration greatly influences the immune response of the fish and the protection offered by the vaccine. Injection vaccination which is usually done by intraperitoneal injection offers the best protection in terms of specific humoral antibody production [3]. However, recent research findings suggest that fish has a well-developed mucosal immunity and exposure of mucosal surfaces stimulates the mucosa associated lymphoid tissue (MALT) viz., gut associated lymphoid tissue (GALT), skin associated lymphoid tissue (SALT) and gill associated lymphoid tissue (GIALT) [4, 5]. Immunization routes exposing the MALT produce better protection to fish at mucosal surfaces which are the natural routes of pathogen entry.

Several routes of vaccination have been studied with varying results. Following are some of the routes of immunization used for fish vaccination.

1. Injection vaccination

Injection vaccines usually produce higher systemic immune response in terms of specific antibody production than other routes of immunization. The duration of immunity is also longer in case of injection probably due to the prolonged release of the antigen when administered along with adjuvants. The size of the fish needs to be 20 g or more for injection vaccination. However injection vaccination causes handling and injection stress to the fish. To minimize the stress fish needs to be anaesthetized before handling and injecting. Fish can be anaesthetized with tricaine methanesulfonate at a dose rate of 100 mg L^{-1} or clove oil at a dose rate of 50 ppm.

Injection vaccination is labour intensive and also requires trained manpower for injecting. Vaccine can be administered by either manually using an automatic syringe which delivers a predefined volume of vaccine for each stroke of the piston or by using an automated system where the fish are fed in a conveyer belt and fish are vaccinated by automatic vaccinators [6]. A trained person can inject about 1000- 1200 fish an hour [7] while an automated system can vaccinate about 7000 to 9000 fish in an hour [8]. However automated system is cost intensive and requires relatively larger fish for vaccination. This restricts its use to farmed high value fish such as salmon and rainbow trout which grow to relatively larger size. Vaccination using an automated system is less stressful to fishes compared to manual injection [9].

Vaccination causes release of corticosteroids immediately after vaccination. Increased corticosteroid levels are found to deplete lymphocyte population in circulation and lymphoid organs and have immunosuppressive effects. However injection vaccination produces significant immune response which overcomes the stress and immunosuppressive effect.

Injection vaccination can be monovalent or multivalent. Multivalent vaccines provide protection to many diseases simultaneously. However fishes respond differently to different vaccine components due to the competition between antigens and some antigens may cause non-specific immunosuppression.

Injection vaccination also produces specific memory and booster doses produce higher antibody levels and protection. Primary vaccination induces T-helper cells which are short lived while subsequent booster doses stimulate long lived memory cells and higher antibody levels although the increase do not match the mammalian immune system. Also the increase in the antibody affinity observed in mammals is not seen in fishes.

2. Immersion Vaccination

Immersion vaccination is the simplest route of vaccine administration. The procedure consists of immersing the fish in diluted vaccine solution for a certain period of time. Immersion vaccine can be administered with minimal handling by reducing the water level in the pond/tank or by transferring the fish to a holding tank. A large number of fish can be vaccinated in a short time with minimal labour involvement. However a large quantity of vaccine is required for the procedure.

Immersion vaccination can be administered by many methods viz., Direct immersion (DI), Hyperosmotic Infiltration (HI), Bath vaccination and Flush vaccination.

i. Direct immersion:

In this method, the fish are collected and held in a holding tank for particular period of time say one hour and then shifted to the culture area. This method is ideal for small fish and is practised before stocking them in the culture ponds.

ii. Hyperosmotic infiltration:

In this method the fish are subjected to a hyperosmotic stress by immersing them briefly in a hypertonic solution like urea or sodium chloride before immersing the fish in the vaccine solution. The vaccine can also be added to the hypertonic solution and the fish may be exposed to the hypertonic solution and vaccine simultaneously. This method is suitable for

small fish just before stocking. However this method causes stress to the fish. Although the increase in immunity and protection offered is variable. In general hypertonic infiltration gives a better protection to the fish compared to DI [10].

iii. Bath vaccination:

This procedure involves lowering the water level in the holding tank/pond and addition of vaccine to the water. This method can be practised for all sizes of fish. However large quantities of vaccines are required. This method is less stressful as there is no handling of fish. Higher dilutions of vaccine require longer bath duration for an effective immune response while for lower dilutions shorter bath duration is sufficient. However studies reveal that higher dilution and longer duration provides better protection than lower dilution and shorter duration [11].

iv. Flush vaccination

This method is similar to bath vaccination, except that the water level is not reduced. This method can be followed for all sizes of fish. However this method is less practised due to the requirement of large volume of vaccine. This method is the least stressful as fish is not handled and there is no change.

v. Spray vaccination

This method involves spraying the vaccine on the fish. The procedure requires less quantity of vaccine and can be practised for larger fishes. This method is usually adopted when fish are shifted within the rearing facility. This method is stressful since the fish has to be netted and carried on a conveyer belt for vaccination [7].

vi. Ultrasound:

This is a technique by which fish are immunised in water containing vaccine and is subjected to high frequency sound waves of about 20 kHz. This enhances the permeability of the cells and uptake of antigen [12]. This technique required less concentration of antigen and is said to give protection comparable to injection vaccination [6]. This method is ideal for administering DNA vaccines.

3. Oral Vaccination

In oral vaccination the vaccine is administered usually though feed. The vaccine is either premixed with feed while preparing the feed or the feed is coated with the vaccine

usually before feeding [6]. Vaccine can also be administered orally by intubation, a technique by which the vaccine dose is directly administered in the pharynx. However this method is used only for experimental trials as it is not practical to administer the vaccine orally to individual fish as it is labour intensive, stressful and may cause mechanical injury in the mouth of the fish [13].

Micro and nano particles can be used to encapsulate or conjugate the antigen. Many micro and nano particles have been experimentally tried and found to be useful for vaccination. Nanoparticles are more efficient in antigen delivery and are more uniform in size. The particles may be natural or synthetic polymers. Particles like alginate, chitosan, polylactic co-glycolic acid (PGLA) are some of the particles used in oral vaccination. These micro and nanoparticles are also useful in delivering DNA vaccines. Chitosan has many advantages being a natural polysaccharide obtained from crustaceans is non-toxic and biodegradable. Many researchers have used chitosan nanoparticles for DNA vaccination successfully. Chitosan particles has the added advantage that they have mucoadhesive properties and thus adhere to the skin and gill mucus enabling effective antigen uptake [6].

4. Anal intubation

Oral vaccination has the disadvantage that the antigen is degraded in the acidic environment in the foregut before it reaches the hindgut where the GALT is located. To overcome vaccine can be administered by anal intubation. In this method the vaccine is administered into the hindgut though anus using a special type of blunt end syringe or a micropipette. The antigen is taken up by the lymphoid tissue and a local mucosal and systemic immunity is developed. However this method has the disadvantage that the fish needs to be handled and anal administration may cause injury if sufficient care is not taken.

Viral vaccines for farmed finfish

Vaccination is an effective method for control of diseases of farmed finfish. A number of vaccines have been developed and are commercially available for the control of viral infections. Most of the vaccines available commercially are inactivated vaccines containing formalin or heat killed virus. These vaccines are mostly administered by intramuscular or intraperitoneal injections and hence they are used for bigger fishes as it is practically not possible to inject smaller fishes.

Since many viral diseases affect early stage of fish, alternate vaccination methods such as immersion and oral vaccines have been developed. Immersion vaccines are easy to

administer especially for smaller fish in large batches without causing much stress to the fish. However repeated booster dose administration is a problem.

Oral vaccines are easy to administer to all size fishes and repeated booster doses can be administered easily. The major concerns of oral vaccines are the stability of the antigen during the storage period and degradation of the antigen in the foregut of the fish. This problem can be overcome to certain extend by micro or nano encapsulation of the antigen in particles such as chitosan, alginate etc. More efforts are required to increase the efficacy of oral vaccines which is a promising method of vaccinating farmed finfish with least stress. Commercially available vaccines for some of the common viral infections are given in table 2.

Table 2: Commercially available viral vaccines for finfish

Name of the product	Type of vaccine	Delivery method	Disease/Pathogen	Target Species	Produced by
AQUAVAC® IPN Oral	Recombinant	Oral vaccine	Infectious pancreatic necrosis (IPN) virus	salmon fry	Merck Animal Health
NORVAX® Compact PD	Inactivated vaccine	Intraperitoneal injection	Salmonid Alphavirus (SAV)/ Pancreas disease	Atlantic salmon	Merck Animal Health
NORVAX® Minova 6	Inactivated, multivalent vaccine	Intraperitoneal injection	furunculosis, classical vibriosis, coldwater vibriosis, wound disease and infectious pancreatic necrosis (IPN)	Atlantic salmon	Merck Animal Health
KV3 Vaccine	Attenuated virus vaccine	Immersion in a tank and Injection	KHV disease	Common Carp and Koi carp	KoVax Ltd., Israel
ALPHA JECT micro® 6	Inactivated, multivalent	Intraperitoneal injection	*Aeromonas salmonicida, Vibrio salmonicida, Listonella anguillarum Moritella viscosa* and IPN	Atlantic salmon	PHARMAQ AS, Norway
ALPHA JECT® 2-2	Inactivated	Intraperitoneal injection	Furunculosis IPN	Atlantic salmon	PHARMAQ AS, Norway
Autogenous VNN vaccine	Inactivated	Intraperitoneal injection	Viral Nervous Necrosis	European Sea bass	PHARMAQ AS, Norway
CIBA-Nodavac-R	Recombinant	Intraperitoneal injection	Viral Nervous Necrosis	Asian Seabass	ICAR-CIBA, Chennai
ALPHA JECT MICRO 1 PD	Inactivated	Intraperitoneal injection	Salmon Pancreas Disease Virus (SPDV)	Atlantic salmon	HPRA, Ireland
ALPHA JECT® micro	Inactivated	Intraperitoneal injection	Infectious salmon anaemia	Atlantic salmon	PHARMAQ AS, Norway

1 ISA					
Subunit vaccine against ISA oral powder	Subunit vaccine	Oral	Infectious salmon anaemia	Atlantic salmon pre-smolt, from 10 g of bodyweight.	Virbac

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Fish feed formulation and preparation for fish farming

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Aquaculture is the fastest growing food producing sector with 8.4% compounded annual growth rate since 1989 and is producing 68.3 million tonnes (mt) (FAO-FISHSTAT 2010). Asia contributes about 91% of the worlds' total aquaculture production with China, India, Japan, the Republic of Korea, the Philippines, Indonesia and Thailand as top producers. Indian aquaculture has demonstrated a six and half fold growth over the last two decades.

The use of formulated feeds have become unavoidable especially under high stocking densities as observed in practices of intensive and semi-intensive rearing of fish and shrimp.

Brackishwater fish species like Asian seabass, mullets, milk fish, pearlspot, *Mystus gulio* need food to get the energy that they requires for movement and all the other activities like growth, reproduction etc. However, they are 'cold-blooded' and they do not therefore have to consume energy to maintain a steady body temperature and they tend to be more efficient users of food than other farm animals. The food requirement of different species finfish vary in quantity and quality according to the nature of the animal, its feeding habits, size, its environment and reproductive state.

Fish diet should have adequate energy, not only to meet the needs of body maintenance called basal metabolism, but also for growth. In nature, fish feeds on a variety of food items and derive their balanced nutrition for healthy growth. When they are cultured in confined pond they should be provided with a balanced diet (complete food) as close to natural food as possible. This is the reason for understanding the nutritional requirement of candidate species.

Balance diet/Complete diet

The food/diet which contains all the nutritional components (nutrients) and can fulfill all the nutritional needs of a particular species on 24 hours basis which in turn promotes best growth and reproduction of the species.

Nutritional requirement of different brackishwater species

Following nutrients (nutritional components) are essentially required in the diet of all fin fish: protein, lipid/fat, carbohydrate/starch, vitamins and minerals.

Protein

Protein is the most important nutrient in the diet of fish. Protein requirement of aquatic organism is higher than terrestrial animals. Fish require food protein in the form of essential amino acids for maintenance of life, growth and reproduction and the requirement of protein depends on animal characteristics i.e., species, physiological stages, size as well as dietary characteristics, i.e., protein quality (digestibility and biological value), energy level etc. Scarcity of carbohydrate and abundance of protein and lipid in the natural aquatic food web is also probably responsible for the common trend of aquatic organisms to use protein as an energy source.

Protein requirement vary with the age of the fish. Younger animal generally require higher levels of protein (5-10% more protein) than older animals. Carnivores require high dietary protein (40-50%) than omnivores (25-35%). Among the brackishwater finfishes, requirement of protein for Asian seabass (*Lates calcarifer*), mullet (*Mugil cephalus*) and nona tangra (*M.gulio*) is 40-45%, 27-35% and 25-30% respectively.

Lipid

Apart from its major role to supply energy lipid also act as precursors to many reactive substances. Phospholipids are responsible for the structure of cell membranes (lipid bi-layer). Fatty acids are the main active components of dietary lipids. Fat levels of 6-8% are adequate in most of the fish diets. However, the quality of fat in terms of fatty acids is more important.Carnivorous fish such as seabass can utilize lipids more effectively and lipid level as high as 20 % can be used in their diet. However, lipid level should be adjusted in diet considering the technological problems in feed manufacture and storage. Fish oil and soya oil are generally used as lipid source during feed formulation.

Fatty acids

Fish are unable to synthesize fatty acids of the n-3 and n-6 series and must be provided in their diets. Aquatic animals require higher n-3 fatty acids than terrestrial animals. Among aquatic animals, marine habitat requires more HUFA than freshwater counterparts. The fatty acids, EPA and DHA, which are known as highly unsaturated fatty acids (HUFA) of n3 series, are particularly important. Fresh water fish show requirement for n6 and n3 essential fatty acids (EFA), whereas marine fish show requirement of n3 and also HUFA. Marine fish oils are rich dietary source of n-3 series while plant oils are rich in n-6 fatty acids.

Carbohydrate

Carbohydrate is an inexpensive source of energy in fish diet. Generally carbohydrate utilization by fish is found to be lower than that of terrestrial animals. Fish can utilize dietary carbohydrate up to 40%. For carnivorous fish carbohydrate level in the diet may be in the range of 10-20 %. Depending upon the total energy content required in the diet, carbohydrate can be used from 10-40% level. Using starch as source of carbohydrate in diet has dual advantage. Besides being energy source, it can act as binder if gelatinized by cooking with moisture and hence improve water stability of diet. Corn flour, wheat flour, tapioca flour and other grain flours are good source of starch in fish diet. Another polysaccharide, cellulose is required in the diet as roughage for improving the feed efficiency in fish and shrimp. Cellulose level in the diet of fish may be up to 10 %.

Mineral requirement

Micronutrient such as vitamins and minerals significantly influence the growth and survival of fish and these cannot be synthesized by these organisms.

Fish can absorb minerals directly from aquatic environment through gills and body surfaces or by drinking. Hence, dietary requirement of minerals is largely dependent on the mineral concentration of the aquatic environment. In saline water, calcium (Ca) is abundant, which is absorbed by most aquatic animals. Since the availability of phosphorus (P) through water medium is poor, P should be made available through diet. Usually the preferred Ca:P ratio is 1:1 in feeds of aquatic species. Mono and dicalcium phosphate contain more available P than tricalcium phosphate. Incorporation of P should be very discrete in fish feed, as most of it gets excreted leading to eutrophication. The dietary requirement of P ranges from 0.5-0.9% in fishe. The requirement of magnesium (Mg) fish ranges between 0.04-0.3%. The requirement of zinc (Zn) ranges from 15-30 mg/kg diet while the requirement of iron (Fe) ranges from 150-200 mg/kg diet.

Vitamin requirement

Micronutrient such as vitamins and mineral significantly influence the growth and survival of fish and this cannot be synthesized by these organisms. Even though, some vitamin such as niacin can be synthesized by number of animals but are typically insufficient to meet physiological demand. Hence, supplementation of vitamins in feed becomes necessary for most of the aquatic organisms. Unlike domestic higher animals, the recommended doses of vitamin for aquatic animals are higher, as many vitamins are lost

during the process of feed manufacture and also due to leaching. Destruction of vitamin-C due to oxidation is one of the biggest problem during feed manufacture. Many fishes cannot synthesize vitamin- C from glucose due to absence of enzyme L-gulonolactone oxidase. Major role of vit C is in the formation and maintenance of intracellular material having collagen or related basal constituents in bones and in soft tissues. Among the 11 water soluble vitamins, three (vit C, inositol and choline) are required in large quantities. Sources of choline include cottonseed meal, fish meal, shrimp meal, soyabean meal and yeast. Stable form of vit C is available commercially.

Different ingredients used for fish/shrimp feed formulation

Wheat flour, rice flour, maize flour, soybean cake, ground nut cake, cotton seed cake, sun flower cake, fish meal, prawn meal, prawn head meal, squilla, squid, clam meal, cuttle fish, meat meal, silk worm pupae meal, shark liver oil, cod liver oil, fish oil, soybean oil, soyalecithin, sunflower oil, safflower oil, brewer's yeast, spirulina, mineral mixture, vitamin supplement and binder (guar gum, cellulose, hemicellulose and synthetic binder) are the ingredients generally available for selection and use for formulation of fish/shrimp feed.

Types of Feed:

Aqua feeds in India evolved commercially in late 90's for shrimp culture and then for fishes.

In the brackishwater aquaculture sector majority of the feed used is scientifically formulated compounded feed produced by multinational / Indian company. In fresh water aquaculture, the traditional farm made feed consisting of rice bran with any of the oil cakes available in their locality is used by most of the farmers.The sinking feed for carps and the recent introduction of floating feeds for fish seems to make a steady and positive impact in changing traditional feeding system and increasing the productivity. Generally farm made feed, sinking pellet and floating feed is used in finfish farming.

Feed Formulation

The main objective or aim of feed formulation is development of a nutritionally balanced mixture of feed stuffs which will be eaten in adequate amounts to provide optimum growth of the cultured fish at an acceptable cost by utilizing knowledge of nutrient requirements, locally available feed ingredients and digestive capacity of the organism. The development of nutritionally balanced feed involves several technical and economical factors -

i) The market value of the species: The value of the species cultured using the feed will set the upper limit which can be spent on the formulation of finished diet. As a thumb rule the total dry diet cost should not exceed 25% of the farm-site price of the cultured species.

ii) Dietary nutrient requirement of the species: Protein, amino acid, fatty acid, mineral, vitamin and energy requirements are different for each stage of culture (larvae or fry, juveniles, growout and broodstock).

iii) Natural feeding habits of the species: If information on the dietary nutrients requirements is not known, analysis of its natural feeding habits in the wild will indicate its position in the aquatic food chain (herbivore, detritivore, omnivore or carnivore), preferred food items and feed size, feeding station (surface, column, bottom) and feeding behaviour (daylight or nocturnal feeder, visual or olfactory feeder, rapid or slow feeder, continuous or intermittent feeder). The natural feeding behaviour will also indicate the preferred physical characteristics of the feed to be produced (such as feed size, shape, texture, palatability, buoyancy and water stability).

iv) Ingredient availability, composition and cost: Seasonal availability of ingredients, proximate composition, digestibility and nutrient availability should be taken into account during selection of ingredients. Constraints of using some feed ingredients having antinutritional factors must also be kept in mind. Additional cost for ingredient handling, processing prior to mixing or pelletizing and transportation cost is also to be considered.

v) Manufacturing process: The formulation will depend upon the manufacturing processes employed (cold pelleting, steam pelleting, expansion pelleting, flaking, micro-encapsulation) and the type of feed to be produced (mash, crumble, paste, ball, moist pellet, dry pellet).

vi) Intended stocking density and farm production system: The stocking density (extensive, semi-intensive or intensive) and production system (tank, concrete pond, earthen pond, pen or floating cages) will determine the availability of natural food organisms in the culture system and whether there is necessity for complete diet or supplementary diet formulation.

Before proceeding with formulating a feed, the ingredients are to be selected from available sources. No single ingredient can be expected to provide all the nutrient requirement. Each ingredient in the diet should be included for a specific reason i.e., either to supply a specific nutrient or physical property to the diet. Formulation of a feed by the nutritionist is only the beginning of a process that ends when the feed is finally consumed.

Feed formulation is essentially a recipe making process keeping in mind the nutritional requirement of particular species, palatability and growth promoting ability of that feed. These objectives can be achieved by judicious selection of feed ingredients, mixing them in proper proportion and presenting them in a most acceptable form.

The basic technique used in ingredient selection is through "Least cost" or "Best buy" calculations

Least-cost or Best-buy technique

The price of the feedstuffs used in diet formulations must be considered to formulate a cost-efficient diet. Feedstuffs can be compared with one another on the basis of cost per unit of protein, energy, or amino acid. The cost of protein is often the greatest part of the cost of a fish diet. Therefore, substantial savings can be made by using best-buy techniques to determinate least expensive protein supplement.

When several feedstuffs are available to supply a particular nutrient then it is useful to calculate the cost per unit of nutrient from each of the ingredients and compare.

Example: If soybean cake costs Rs.45/kg and contains 45 % protein-

Cost/ kg protein = 45/0.45 = Rs. 100

Ground nut cake costs Rs. 35/kg and contains 40 % protein

Cost/kg protein = 35/0.40 = Rs.87.50

Thus, although soybean cake contains higher level of protein, the cost per kg protein from ground nut cake is less. Therefore ground nut cake is a better buy.

To compare feedstuffs on the basis of cost per unit of an amino acid, one can calculate the best buy in the same way as before. For example, sesame oil cake which has twice as much methionine content as does groundnut cake on a per unit protein basis would be a more attractive buy at comparable prices. These kinds of comparisons are only valid if the nutrient in one feedstuff is as valuable or available to the animals as the same nutrient in another feed. Such comparisons should be made whenever prices charge.

Balancing nutrient levels

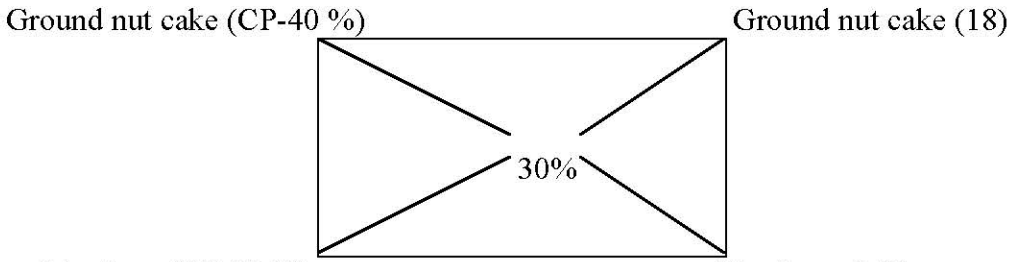
In most animal diets, protein is the most expensive portion and is usually the first nutrient that is computed and balanced in diet formulation. The energy level of the diet is then adjusted to the desired level by addition of high energy supplements which are less

expensive than protein supplements. The square method is an easy way to determine the proper dietary proportions of high and low protein feedstuffs to add to a feed to meet the dietary requirement of the animal to be fed. The protein in the diet can be adjusted by following Pearson's square method.

The protein in the diet can be adjusted by following Pearson's square method.

For example 1.

To prepare a feed with 30% protein using ground nut cake (CP – 40 %) and rice bran (CP-12%), a square is constructed first and the names of the feed ingredients with protein percentage are written on the two left corners. The required protein level of feed is written in the middle of the square. Next, the protein level of the feed is subtracted from that of the ingredients in corner wise and answer is placed ignoring the positive or negative sign.



28

Add the figures on the right hand side of the square, i.e., 18+ 10 = 28

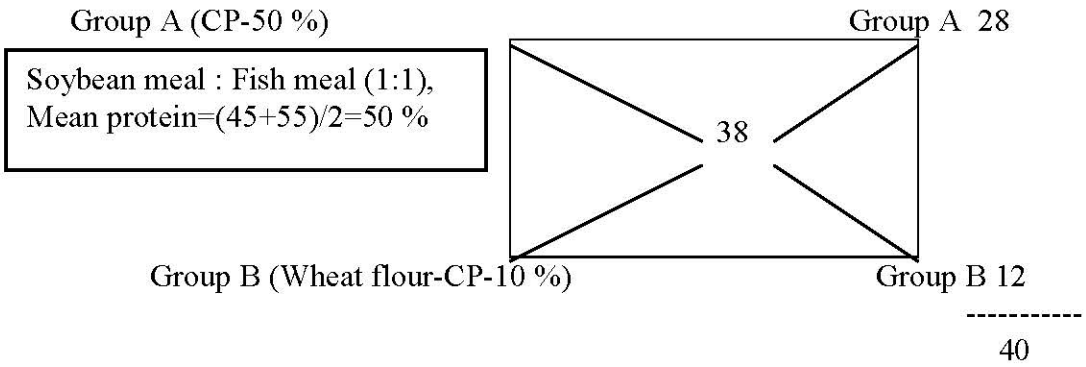
Now to make the feed with 30 % protein we should mix

Ground nut cake- 18/28 x 100 = 64.29 %

Rice bran - 10/28 x 100 = 35.71 %

Example-2. Computing with more than one ingredients

For example to prepare a diet with 38% protein using soybean meal (CP – 45 %), fish meal (CP-55%) and wheat flour (CP-10 %), ingredients are to be divided into two groups- Group A- protein rich ingredients (soybean meal and fish meal) and group B-energy rich ingredient (wheat flour). Mean protein percent has to be calculated from both the groups. A square is constructed first and the names of the feed groups are written on the two left corners along with the mean protein content of each group assuming that under each group ingredients are mixed in equal proportion. The required protein level of feed is written in the middle of the square. Next, the protein level of the feed is subtracted from that of the ingredients and answer is placed ignoring the positive or negative sign.



Add the figures on the right hand side of the square, i.e., 28+ 12 = 40

Now to make the feed with 38 % protein we should mix

Group A ingredients - 28/40 x 100 = 70 %

So, Soybean meal to be mixed - 70/2= 35 %

Fish meal to be mixed - 70/2 = 35 %

Group B ingredient i.e wheat flour - 12/40 x 100 = 30 %

The square method is helpful to novice feed formulators because it can get them started in diet formulation without the need to resort to trial and error. The square method can also be used to calculate the proportion of feed stuffs to mix together to achieve a desired dietary energy level as well as a crude protein level. The square method cannot be used to simultaneously solve for both crude protein level and ME level.

Linear Programming

The mathematical technique available to nutritionists for selecting the best combination of feed ingredients to formulate diets at the least possible cost is linear programming. The informations necessary for feed formulation using linear programming are

1. Nutrient content and DE or ME of ingredients;
2. Unit price of feedstuffs including vitamin and mineral mixtures;
3. Any other additives to be used in the feed; and
4. Minimum and Maximum restriction on the amounts of each ingredient in the feed

Least-cost linear programming software for diet formulation is readily available, the price varying with the sophistication required. A commonly used spreadsheet a such as Lotus 1-2-3 can also be utilized for formulating feeds, incorporating a smaller number of variables. It should be noted that least-cost feed formulation is not always practical for small scale aquaculturists using on-farm feed manufacture facilities where are the choice of ingredients available is limited.

Quadratic programming formulation

Nutrient requirements used in linear programming feed formulation are fixed usually for maximum rate of growth. This may not be the best decision from economic point of view.

Nutrient constraints may be relaxed to bring down feed cost while still achieving acceptable lower growth. Quadratic programming takes into account the growth response within a range of nutrient constraint. Therefore, good understandings of biological response functions from actual feeding trials are essential in the use of quadratic programming. For example, it was reported that inclusion level of arginine could be reduced by 20% with only a 5% likely reduction of growth of Nile tilapia.

Table 1. Proximate composition of ingredients of plant origin

Ingredients	As % dry matter			
	Crude Protein	Crude Lipid	Crude Fibre	Total Ash
Soybean cake	42-48	2-7	6-8	5-7
Ground nut cake	40-43	3-8	6-9	4-8
Gingelly oil cake	32-44	12-16	15-23	11-12
Cotton seed cake	36-44	4-8	16-22	6-9
Sun flower oil cake	38-47	4-6	14-16	6-7
Coconut oil cake	22-28	6-9	12-14	18-22
Deoiled rice bran	15-17	1-2	14-16	16-20
Rice bran	12-14	5-9	15-20	14-28
Wheat flour	9-12	3-4	6-8	4-6
Wheat bran	12-14	6-8	10-14	6-8
Maize	10-12	4-6	4-8	6-9
Sorghum	8-10	2-5	2-3	2-4
Horse gram	16-20	1-2	6-8	7-9
Tapioca	1.5-2.5	0.4-0.6	2-6	2-4
Alfalfa	16-24	2-4	16-30	10-16
Brewer's yeast	40-45	1	2-7	6-9
Spirulina	55-68	6-8	1-3	8-10

Table 2. Proximate composition of ingredients of animal origin

Ingredients	As % dry matter			
	Crude Protein	Crude Lipid	Crude Fibre	Total ash
Fish meal	52-60	5-12	1-4	22-38
Prawn meal	58-65	4-7	4-7	21-26
Prawn head meal	34-45	4-7	11-18	36-44
Squilla	37	4	-	23
Clam meal	40-58	6-12	-	5-9
Cuttle fish	67	5	1	12
Slaughter house waste	54-65	9-26	-	6-18
Blood meal	79-88	1-2	-	4-6
Meat meal	44-52	8-11	2-4	24-31
Poultry waste	52-56	16-35	-	9-18
Silk worm pupae meal	48-53	26-30	6-8	7-11

Table 3. Ingredients used for mineral premix

Mineral	Ingredients Used
Calcium	Calcium carbonate, Calcium phosphate, Dicalcium phosphate, Calcium lactate
Phosphorous	Monosodium,potassium or calcium phosphate, Dicalcium phosphate
Magnesium	Magnesium carbonate, Magnesium sulphate
Sodium	Sodium chloride
Potassium	Potassium chloride, Potassium sulphate
Zinc	Zinc sulphate, Zinc oxide
Copper	Copper sulphate, Copper oxide
Manganese	Manganese sulphate, Manganous oxide
Iron	Ferrous sulphate, Ferrous gluconate, Ferrous carbonate
Iodine	Potassium iodide, Potassium iodate, Ethylene diaminedihydro iodide
Cobalt	Cobalt chloride, Cobalt sulphate
Selenium	Sodium selenite

Feed Preparation:

To prepare a batch of shrimp/fish feed using small scale feed mill equipmenmt, the following methods is to be followed-

Grinding: First grind individually dry fish, prawn head waste, squid waste, soybean meal etc. in a hammer mill to reduce the particle size. Then powder all the ingredients in a pulverizer (atta chakki) separately. For further reduction of particle size micropulverized is used.

Mixing: Weigh the feed ingredients as per the formula and put in the mixer (or hand mixing) except vitamin and mineral mixture. Homogenize the feed mix for 15 minutes. Add 35 litres of water and further homogenize for another 10 minutes.

Steam cooking: Load feed mix in trays and keep the trays in steaming chamber. If steaming chamber is not available, cook the mixture in a big container at 100 ^{o}C temperature for 5-10 minutes. Take out and cool the feed mixture.

Incorporation of Vitamin-Mineral mixture: Add the vitamin and mineral mixture to the steamed and cooled feed mix and thoroughly homogenize in a dough mixer.

Pelletisation: Pelletize the feed in a pelletizer fixed with desired size die. Collect the pellets in aluminium trays.

Drying: Restore the trays loaded with moist feed into an electrical tray drier. Adjust the temperature at 60-70 ^{o} C and allow the feed to dry until the moisture content is less than 10%. Sun dry the moist pellet where electrical drier facility is not available.

Checking quality of feed: Dried pellet feed should be physically examined for visual appearance such as uniformity, color and smell. The pellet should have surface without cracks. Feed may be sampled and analysed for proximate composition. Water stability of the pellet may also be tested after 24 h of preparation.

Storing: Dried pellet feed should be packed properly in polythene bags and kept on raised wooden platform to avoid absorption of moisture and to be used within 3-4 months period.

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Feed management strategy for sustainable brackishwater aquaculture

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1. Introduction

Indian brackishwater aquaculture is the economic engine of Indian aquafarming sector, shown phenomenal growth contributing around 7.92 lakh tons with major share of 6.92 lakh tons by shrimp production (MPEDA, 2019). Scientific aquaculture farm management in brackishwater sector in India was started during early 1990s with major focus on the indigenous black tiger shrimp (*Penaeus monodon*). Besides tiger shrimp, certain marine/ brackishwater fishes such as seabass, milkfish, mullets, catfish and pearlspot have shown enormous potential for commercial aquaculture. Since 2009, the culture of exotic white-leg shrimp, *Penaeus vannamei*, has become major brackishwater species, occupying more than 90 % of total shrimp production of India, because of its faster growth rate, better feed utilization efficiency, availability of Specific Pathogen Free (SPF) broodstock, and culture possibility in wide range of salinity.

Feed is a major input in Brackishwater aquafarming contributing 60-70 % of operational expenses. Preparation of nutritionally adequate aquafeed involves understanding the dietary requirements of the species, proper selection of feed ingredients, formulation of feeds and appropriate processing technology for producing water stable pellet feeds. Depending upon the type of farming, a wide range of feedstuffs is used for feeding stocked fish and shrimp. While no or little feed is used in traditional farming systems, supplementary feed at adequate level are essential in commercial aquaculture.

The performance and success of a formulated diet depends on many factors, the most important being

i) Feed formulation and nutrient content of feed ingredients
ii) Feed manufacturing process and physical characters of the feed
iii) Feed handling and storage
iv) Feeding strategies-type of feed, feed application methods, feeding regime
v) Aquatic environment and natural food availability

Though scientific feed formulation and manufacturing of quality feed plays major role for growth of any culture species, but proper feed management in culture system is most important to make the culture successful and economic.

Feed management means use of feed in such a way that utilization of feed is optimum; wastage is minimum thereby negligible impact on environment, achieving best feed conversion ratio and maximum growth and production of fish and shrimp. A very good quality feed can produce poor result if the feed management is poor, whereas, a moderate feed can produce very good results under good feed management.

The foremost critical factor is selection of appropriate feeds and planning of optimal feeding regimens. Suitable feed should fulfill the nutritional requirements of species under culture. Proteins, lipids, carbohydrates, vitamins, minerals and water are the six major classes of nutrients, which are used for building, maintenance of tissues and supply of energy. The requirement for these nutrients varies depending on the species according to their feeding habit, habitat in which they live in and the stage in their life cycle. Our aim should therefore be to produce nutritionally balanced feed with optimum protein energy ratio. It should also ensure that nutrients are not lost in water during the feeding process. Therefore, aquaculture feeds of different formulations are processed using the special technologies to ensure the diet remains intact in water before ingestion, and nutrients are prevented from dissolving. These general categories of feeds used in aquaculture are wet feeds with moisture contents of 50-70 percent, semi moist formulated feed with moisture contents of 20-40 percent and dry pelleted feeds with moisture contents of less than 10 percents. Since problems are associated with the distribution, handling, utilization, storage and quality of wet feeds and moist feeds, more and more dry feeds are manufactured either by steam pelleting or by extrusion pelleting. Feeding management for fish and shellfish are quite different and discussed below.

2. Optimizing feed management strategies

The cost-effectiveness of aquaculture operation is of supreme importance to the farmer. Adopting proper feed management strategies is the key in ensuring that feed use is optimum and that the highest net profit are available to the farmer (FAO, 2010). While maximum growth rates can be attained by feeding to satiation, over- or under-feeding may result in feed inefficiencies (Kaushik, 2000) and, in the case of over-feeding, increased levels of farm effluents. Optimization of feeding strategies requires farmers to calculate appropriate ration sizes and feeding rates, feeding frequencies, and feeding times that take into

consideration the endogenous feeding rhythms of the farmed species. Farmers those are using commercially manufactured feeds are often but not always supplied with feeding tables, and are provided with technical support to assist them in determining ration sizes and feeding schedules. In many respects it is in the interest of the feed manufacturing company to ensure that their feeds are used appropriately - it promotes good production outcomes for the farmers and enables them to develop long term commercial relationships. Those farmers that are using farm-made feeds and purchase feed ingredients from suppliers are less likely to have access to the information to determine the feeding regimes. In the absence of this information, farmers will find it difficult to determine appropriate feed rations, and in many respects, they are more likely to adopt inappropriate feeding strategies. Sometimes farmers failed to take into consideration ambient temperature, body mass and pond biomass when determining feed rations. In the absence of this data it is difficult for farmers to optimize strategies to feed their animal to improve on production efficiencies. There is a clear need to train farmers in feed management practices, promote the use of feed tables and ensure that farmers maintain adequate feed and production records.

3. Feeding management in fish culture systems

Like other animal husbandry practices, feeding is most important and crucial for viability and success in aquaculture. Following points should be strictly followed while feeding the fish for maintaining good pond hygiene and to reduce wastage of feed and to avoid accumulation in pond bottom.

1. Pond biomass Estimation: Pond biomass should be assessed regularly and ration size should be determined as per biomass of the pond.
2. Number of times organism should be fed in a day i.e., frequency of feeding
3. When feed should be offered i.e. time of feeding
4. The way feed should be offered in the system i.e., method of feeding.

3.1. Ration size

Ration size is the allotted feed quantity for the cultured species for the period of 24 hours. Unlike other terrestrial animals, optimum quantity of feed determination is very difficult task in aquaculture opearation. The size of daily food ration, the frequency and timing of meals are the key factors influencing the growth and feed conversion. Hence, the optimal feeding regimens must be determined as per the feeding behavior, appetite and functioning of the digestive systems. Fish lose weight when their food intake falls below that

required for maintenance. When ration size increases, the growth rate increases. But over feeding in aquaculture leads to wastage of feed and deterioration of water quality and thereby affect growth of cultured species though feed may be of very good quality.

Ration size may vary depending on life stages of fish. Young fish require higher energy for metabolism per unit weight for their faster growth than an adult fish which require comparatively less energy for their maintenance. Thus young fish require bigger ration size. The quantity of ration varies from 100% of body weight for larvae and fry and gradually reduced to 50 %, 20%, 10%, 5% and 2-3% as the fish/shrimp grow marketable size. Ration size may also vary because of physiological stress created due to fluctuation in water quality parameters, particularly temperature. Water temperature higher or lower than the optimal range may affect the feed intake and hence ration size needs to be adjusted to minimize the feed wastage. Generally the method of calculating the daily ration is based on the body weight of fish.

$$\text{Ration size (Kg)} = \frac{\text{ABW(g)} \times \text{Stocked nos.} \times \text{Survival(\%)} \times \text{Rate of feeding(\%)} \times 1\text{kg}}{1000 \times 100}$$

Ration size is also estimated by various methods using the feeding charts, feed equations, growth prediction and check tray etc. Feeding rates for seabass of 1-2 g is 20 %, 2 -20g is 15-7 %; 21-55 g is 6-4 %, 56-180 g is 4-2 % and when body weight is more than 180 g feeding rate is 2 % of biomass (De et al. 2012). For assessing the average body weight, sampling needs to be done on regular basis i.e., weekly or fortnightly to recalculate the amount of feed to be given. Use of sensor based demand feeder may help for precise estimation of ration size which may be helpful in large scale farming. Although the total quantity of feed needed will increase as fish grow, the amount of feed offered as a percentage of total fish biomass needs to decrease over time. Besides the ration size, the optimal feed particle size also affects the growth and feed conversion efficiency. Large fish can ingest small particles, but it requires more energy to capture the required equivalent weight or smaller food particles. This results in measurable reduction in food conversion efficiency (Goddard, 1996). Attention should also be given to the influences of feed shapes, colors and textures of pellets on ingestion rates.

3.2. Feeding Frequency and Time of feeding

Feeding frequency is very important to increase feed utilization efficiency and to reduce the feed conversion ratio and to ensure maximum dressing percentage of cultured

organism. Determination of feeding frequency is a researchable area in aqua nutrition and it differs from species to species and culture system. Species which require more time for evacuation of stomach require less number of feeding compared to species in which passage rate of feed in gastrointestinal tract is faster. Generally young fish are fed more often and the frequency of feeding decreases as the fish grow. As a thumb rule fish should be fed at 1 % of body at each meal. For example if the ration size is 5 % of biomass, fish should be fed five times each at 1 % of biomass. Most of the Brackishwater fishes are fed 3-4 times a day. Higher feeding frequency reduces starvation and stunting and results in uniform growth of fish. The total feed required in a day should not be fed at a time. Scheduling and frequency of feeding greatly help in successful feed management. Time schedule for feeding the fish may be fixed in such a way that larger ration may be given when the fish is expected to be most hungry. There should be a minimum of three time schedules of feeding in a day- morning, noon and evening. Lighting regime also influence the feeding frequency in fish and research data indicates 24 h lighting regime during initial period helps to improve feed intake and feeding frequency may be upto 20 times per day. In brackishwater polyculture, study suggests that daily ration has to be distributed at three equal quantities in the morning (09:00 hours), afternoon (13:00 hours) and evening (17:00 hours). Total feed has to be offered in two split doses at 1 h intervals (09:00, 10:00 hours), (13:00, 14:00 hours) and (17:00, 18:00 hours) to meet the requirements of fish with various size-groups (De et al., 2018). Frequent feeding of small portion of ration help in better utilization of the feed and thereby lead to efficient FCR. There must also be a mechanism in each case to monitor the feed consumption and offering of next dose of feed should be regulated on basis of consumption from the previous feed offered. In fish, 90 % of offered feed should be eaten within 15-30 minutes of feeding time.

3.3. Feeding methods

Feeding method is one of the important aspects for cost effective production of high quality fish. Depending on different factors such as labour costs, scale of farming, species under farming, type of system i.e. for hatchery or grow out systems, fish can be fed through hand feeding or mechanized feeding. Generally farmers feed their fish by directly broadcasting the feed in pond (Fig. 1). Broadcasting or hand feeding is the most common form of feeding in the semi-intensive culture practices of the developing world. It is also used in intensive culture practices to varying extent. Feed bag with few small hole suspended at different places in ponds is also common method of feeding for fish (Fig 2 & 3). For

improving feed utilization efficiency floating feed can be given in net enclosure (Fig 4) and sinking pellet may be given in feed tray (Fig 5). Finfish are easily conditioned to feeding and react to the first appearance of food, making them suitable for broadcast feeding (De Silva & Anderson, 1995). Sometimes feed are offered in the dough form kept on a platform just below the water surface. For Asian seabass, primarily fry should be weaned with formulated feed in hapa, cement cistern or fibre tank (Fig. 6) so that feeding can be monitored easily. They should be fed with little patience as it takes longer time to feed (De et al., 2012). In grow out pond weaned seabass are offered feed by broadcasting the feed in 4-6 places in 1400 sq m area. Feed should be broadcasted as long as fishes stop taking the feed. When the fishes are habituated with pellet feed they should be transferred to culture pond.Sampling should be done periodically for estimation of biomass. Feeding should be monitored carefully. Soil and water monitoring should be done at regular interval. Often farmers use a combination of feeding methods such as hand feeding to mechanized feeding. In mechanical feeding system, demand feeder is used in which fish approaches to the feeder for its feed requirements when they feel hungry. It was observed that fish quickly learn how to obtain feed. The growth of fish is good with best FCR and minimum wastage of feed in self-demand feeding system. This method works best with finfish farming. A reliable and least- cost feeding system should ensure the effective distribution and spread of adequate feeds in aquaculture ponds.

Fig. 1. Rope bag feeding method

Fig. 2. Pole bag feeding method

Fig. 3. Feeding in net enclosure

Fig. 4. Feed broadcasting in pond

Fig. 5. Feeding in feed tray

Fig.6. Weaning of Asian seabass with formulated feed in tank and hapa

4. Feeding management in shrimp culture system

Proper feed management is essential for successful and profitable shrimp farming. As feed alone costs 50-55% of total culture expenditure, strict supervision on feeding is required. Shrimp find their feed mainly by chemosensory mechanism rather than vision. The chemoreceptors are concentrated on the anterior appendages, antennae and antennules. Once the smell is detected, the shrimps move towards the feed and quickly grasp it with their chelate pereiopods. As the shrimps are sluggish feeders, it is practically difficult to feed them up to their satiation. Hence, shrimps are fed following prescribed schedule based on culture system. Due to benthic feeding behavior, sinking pellet feed should be offered to shrimp. Following points should be strictly followed while feeding the shrimp for maintaining good pond hygiene and to reduce wastage of feed and to avoid accumulation in pond bottom.

1) Proper feeding guidelines should be followed to fix ration size for shrimp culture pond
2) High quality feed should be used
3) Daily ration should be offered in 4/5 meals

4) Feed intake should be checked through check trays (6 nos/ha)

5) Feed should be reduced up to 50% during major moulting

6) Feeding should be avoided during heavy rain

7) Feed of proper pellet size should be offered

Appetite of shrimp will vary due to the environmental conditions i.e., water quality, water temperature, sunny/overcast days and physiological conditions such as disease and moulting. Feed should never be given in excess as uneaten feed pollutes the water. As shrimps are the nocturnal feeder, larger doses may be offered in the evening and during night. Unlike other shrimps, white leg shrimp (*Penaeus vannamei*) are more active during day time and hence major share of the ration is offered during day time. Generally during new moon and full moon moulting of shrimp takes place and they become physiologically less active and reduce the feed intake. Quantity of feed offered should be reduced at the extent of 30-50% during that period. Regular observations and experience helps in mastering the management of feeding in a culture farm.

4.1. Ration size, Feeding frequency and Time of feeding

Generally the method of calculating the daily ration is based on the body weight of shrimp (Table 5 & 7). Blind feeding is generally practiced during first fifty days of culture (Table 4 & 5). Daily ration is divided and offered 2 to 5 times a day (Table 6 & 8) depending on stages of culture. The feeding activity and quantity of feed consumed may be checked by keeping feed in check trays (size: 80 cm x 80 cm) @ 6 nos./ha in different places in pond. After one month of stocking, consumption of feed should be checked by using check trays. Besides the ration size, the optimal feed particle size also affects the feed intake and growth of shrimp. Feed particle size should vary as per body weight of shrimp (Table 9). Feed should be broadcasted evenly in a periphery of about 2 meters from dyke in all sides of the pond.

Table 4. Ration size for first fifty days of tiger shrimp farming

Age (Days)	Feed increment / day (g)	No. of meals / day	Feed (Kg) / day / lakh PL_{20}
1	-	2	2.0
2-10	400	2	2.4-5.6
11-30	600	3	6.2-17.6
31-50	500	4	18.1-27.6

Table 5. Ration size after 50 days of culture in tiger shrimp based on check tray performance

Days of culture	Expected ABW (g)	% of biomass as feed	Feed % in Check tray	No. of meals / day
51-55	6-7	5.0-4.8	2.0	4
56-60	7-8	4.8-4.6	2.2	4
61-65	8-9	4.6-4.4	2.2	4
66-70	9-10	4.4-4.2	2.4	4
71-77	10-12	4.2-4.0	2.6	4
78-83	12-14	4.0-3.7	2.7	4
84-90	14-16	3.7-3.5	2.8	4
91-97	16-18	3.5-3.2	2.9	4
98-104	18-21	3.2-2.9	3.0	4
105-110	21-24	2.9-2.7	3.2	4
111-117	24-27	2.7-2.5	3.3	5
118-124	27-30	2.5-2.2	3.5	5
125-131	30-33	2.2-2.0	3.6	5
131-133	33-36	2.0-1.8	3.7	5

Table 6. Feeding Schedule for tiger shrimp

Feed type	Shrimp weight (g)	Time of feeding				
		6.00 AM	11.00 AM	6.00 PM	10.00 PM	2.00AM
Starter	Up to 4.0	30 %	-	35%	35 %	-
Grower	4 – 25	25 %	15 %	30 %	30 %	-
Finisher	> 25	25 %	15 %	20 %	25%	15%

Table 7. Ration size for *Penaeus vannamei*

Age in days	Feed increment / day	Feed(kg) / day / lakh PL_{15}
1	-	2.0
2-10	400	2.4-5.6
11-20	500	6.1-10.1
21-30	600	10.7-15.5
31-50	700	16.2-28.8
After 50 days Body wt of shrimp	% of biomass	Feed in Check tray (g/kg/tray)
5-10	5.5-4.5	2.4-2.8
10-15	4.5-4.0	2.8-3.0
15-20	4.0-3.5	3.0-3.3
20-25	3.0-2.5	3.3-3.6
25-30	2.5-2.0	3.6-4.1

Table 8. Feeding schedule in *Penaeus vannamei* farming

	Percentage of daily ration in meals				
	6 AM	9 AM	12 PM	3PM	6 PM
1st month	40	-	-	60	-
2nd month	40	-	-	30	30
3rd month	20	20	-	30	30
4th and 5th months	15	15	10	25	35

Table 9. Recommended pellet size for tiger/white leg shrimp

Feed type	Size of shrimp (g)	Pellet size
Starter	0-4.0	0.5-1.0 mm crumble
Grower	4.0-25.0	2 - 2.3 mm x 4 - 5 mm
Finisher	>25	2-2.5 mm x 6 – 8 mm

4.2. Check tray monitoring

Quantity of feed to be kept in check tray depend upon pond size and average body weight of shrimp and can be determined using the following formula

$$\text{Quantity of feed (g) in each check tray} = \frac{1600}{\text{Area of pond}} \times \frac{\text{Feed \% in check tray}}{100} \times \text{Quantity of feed in a meal (g)}$$

The check trays should be observed after 2 hr of feeding .Depending on the quantity of feed consumed in the check tray, the next dose should be increased or decreased. Special care should be taken during moulting, shortage of dissolved oxygen and stressed condition due to heavy rain, high temperature, unfavourable pond bottom and water quality. Feed adjustment for shrimp should be done by check tray observation (Table 10).

Table 10. Feed adjustment for shrimp by check tray observation

Average amount of unconsumed feed remaining in trays (%)	Feed adjustment in subsequent meal
0 (zero)	5% increment
<5	No change
5-10	5% reduction
10-25	10% reduction

If tray monitoring is done properly and check tray feed is consumed within 2 hours, survival % can be accurately estimated by the following formula

$$\text{Survival \%} = \frac{\text{Total quantity of feed consumed per day (g)}}{\text{Stocked shrimp} \times \text{ABW (g)} \times \text{\% ABW feed}} \times 100$$

For example in a culture pond of 1500 sq m having total stocked shrimp of 45000 nos consuming 18.225 kg per day and body weight of shrimp is 10 g, assuming feed requirement of 4.5 % ABW, survival % would be

$$\text{Survival \%} = \frac{18225}{45000 \times 10 \times 0.045} \times 100 = 90\ \%$$

Success of feed management depends on the farmer's experience and observation on the feeding behaviour and feed intake of shrimp. Following a strict feed management, tiger shrimp can attain average weight of 30-35 g with survival up to 70-80 % in culture duration of 120 days, whereas exotic white leg shrimp could achieve 20-25 g with a survival of 80-90% in 100 days culture period. Progressive farmers may form co-operative society and can have small scale feed mill to prepare shrimp feed using locally available feed ingredients for tiger shrimp/ vannamei shrimp/Indian white shrimp culture and may get a good economic return.

5. Natural productivity and the implications for feed management

Natural productivity of culture system contribute significant amount of nutrition to farmed fish and shrimp. The levels of natural productivity in ponds can be assessed with simple methods which can help the farmers to manage phytoplankton, zooplankton, and benthos and periphyton production through appropriate use of fertilizer which would increase the pond production efficiencies. Though quantification of nutrient contribution of the natural productivity to the farmed animal is complicated but its assessment may help to minimize the use of supplementary feed in extensive and semi intensive culture system. Feeds often play a dual role by providing nutrition to the animals being farmed and as a nutrient source to stimulate natural productivity. Developing a better understanding of these dynamics is crucial for improvement of nutrient retention in the farmed species which could reduce feed requirement with higher economic return.

6. Handling and storage of feeds

Optimizing handling and storage procedures on farms is an essential component of good management practice. As feeds are composed of perishable biological material, it is

prone to deterioration during storage. Therefore, it is always desirable to minimize storage time. High quality feed can readily spoil and denature if stored under inadequate conditions or for too long a period. Deterioration of feed during storage may be due to oxidative damage, microbial damage, damage due to insect or rodents and other chemical changes during storage. Generally, feed should be stored in such a manner so that feedbags do not touch the floor or side walls. The store needs to be 100% water proof and a damp-proof storage facility is ideal. Feed stacks should be arranged in such a manner that earlier prepared feed should be accessed readily and fed first. Proper ventilation throughout the sorage period is most desirable. Incorrectly stored feeds may not only be unappetizing to fish/shrimp or lacking in essential nutrients but also may contain toxic and antinutritional factors. This can lead to abnormal behaviour, poor feeding response and growth. Hence different feed types such as wet feeds, moist feeds and dry feeds must be handled and stored under appropriate conditions. Wet feed having moisture content of more than 70% should ideally be stored at temperature of -30°C or lower. Common freezer temperature of -20° C are inadequate for long term storage. Dry feed should be stored under cool, dry condition, ideally at temperature below 20 °C and at relative humidity of below 75 %. A normal storage time of 1-2 months is recommended for dry feeds stored in tropical condition (Table 11).

Table 11. Maximum permissible storage time for different feed stuff

Feed stuff	Storage time
Ground ingredients	1-2 months
Whole grain and oil cakes	3-4 months
Compounded dry feed	1-2 months
Vitamin mix. (kept cool)	6 months

Source: New (1987)

Improper storage may lead to oxidation of fatty acids in feed resulting in rancidity. Poly unsaturated fatty acids and pure lipid are very much prone to oxidation. Rancid fat results in less palatability and may produce toxic peroxide compound that inhibits growth of fish/shrimp.Chemicals produced in the deteriorated feed may reduce availability of amino acids and vitamins, vitamin C being most susceptible. One thing to remember that storage never enhances feed quality, but proper storage reduces the rate of deterioration of feed.

7. Water quality

The interrelationships between feeding and water quality in aquaculture is complex. By providing optimal species-specific requirements such as temperature, dissolved oxygen,

pH and salinity, adequate feeding to satiation, improved growth and high survival can be ensured. When the water quality parameters fall below optimal levels, the species under culture will be stressed and feed consumption will be impaired and growth will be affected. Accumulation of left over feed together with excretory products leads to high BOD, NH_3, H_2S, CH_4 and harmful effects of eutrophication is observed. This is a critical issue in management since effluent quality can be linked directly to feeds and feeding practices and is regulated under water pollution control laws in many countries. Thus, feeding regimes should be designed to minimize the nutrient loss and faecal output and to maximize the nutrient retention and health status of the cultured fishes.

8. Conclusion

Judicious feed management is important factor in achieving good feed efficiency and reducing feed wastage. Freshly prepared good quality feed proven with best potential FCR, could reduce feed waste. Feed with poor water stability, which have lost their nutritional potency and are poorly accepted by the fish or shrimp should be rejected. Appropriate particle size of the feed should be designed for a particular stage of life cycle. The ration size and feeding schedules should be regulated with reference to feeding guides, response of fish and environmental conditions.

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21

Use of nutraceuticals in promoting growth and disease resistance in fish culture

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With the intensification of cultural practices, diseases have become the primary constraint in aquaculture. Cultured fishes are susceptible to various kinds of bacterial, fungal, viral, parasitic and nutritional diseases, which impedes aquaculture production, economic and social advancement in the country. There is a constant need to increase productivity in aquaculture, particularly to improve growth rate, feed utilization as well as stress resistance of fish. Because of consumer concerns and strict regulations in many countries, the use of synthetic chemicals, hormones and antibiotics is becoming unviable and natural compounds are more acceptable to the public.

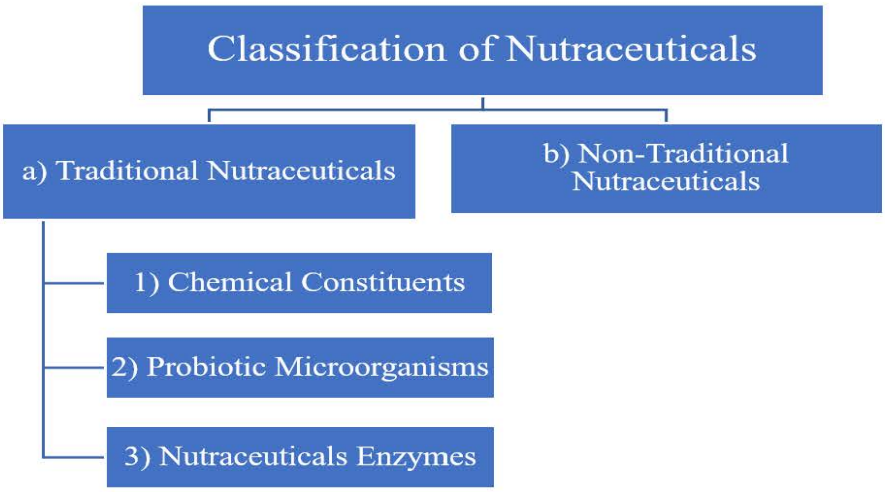
The term "nutraceutical" derived from the terms nutrition and pharmaceutical was coined by Dr. Stephen De Felice in 1989. Nutraceuticals are a group of products that are more than food but less than pharmaceuticals. The term is referred for a nutritional supplement that is used as treatment or to prevent disease. Nutraceutical is a substance that may be considered a food or part of a food which provides health benefits, which enhance prevention and treatment of disease. The pharmaceutical companies have promoted benefits of natural products in the form of capsules and tablets and sold it as nutraceuticals. This can be called as "medicalization in nutrition" where the desired nutrients are acquired in the form of tablets, capsules, or drinks rather than natural food items. Nutraceuticals includes diverse products such as isolated nutrients, dietary supplements and diets to genetically engineered foods, herbal products, and processed foods like cereals, soups, beverages.

Classification

Nutraceuticals or functional foods can be classified on the basis of their sources: natural or traditional and unnatural or non-traditional.

A. Traditional Nutraceuticals

They are natural products with no changes to the food. They contain numerous natural components that convey benefits beyond basic nutrition, like omega-3 fatty acids in salmon, saponins in soy or lycopene in tomatoes.



1. Chemical Constituents

a. Nutrients

Vitamins, minerals, proteins and essential fatty acids are essential nutrients of fish food. Nutritional status is considered one of the important factors that determine the ability of fish to resist diseases. Balance between macro and micronutrients, including amino acids, polyunsaturated fatty acids (PUFA), vitamins and trace elements, perform specific and essential role in development of immune system at the larval stage and maintain optimal health of larvae as well as bigger fish and shrimp. Eicosanoids involved in the regulation of the immune system by their direct effects on cells such as macrophages and lymphocytes or their indirect effects via cytokines.

b. Herbals

Herbs such as olive leaf acts as an antiviral agent by inhibiting or blocking the transcription of the virus to reduce the replication in the host cells and enhance the non-specific immunity. The herbal extracts involve the fungal cell wall lysis, altering the permeability, affecting the metabolism and RNA and protein syntheisis. Plant Datura metel shows antifungal properties against *Aspergillus* and *Candida*. Plants like Neem (*Azadirachta siamensis*) and tea seed oil (*Melaleuca alternifloria*) shows antifungal properties against *Aphanomyces invadans*. Herbs like *Picrorhiza kurroa* is used as the anti-stress compound for shrimps. The antibacterial properties of herbs include lyse the cell wall, block the protein synthesis and DNA synthesis, inhibit the enzyme secretions, interfere with the signaling mechanism of quorum sensing pathway. Neem leaves, garlic and turmeric powder produce disease resistant fry of Indian major carps.

c. Phytochemicals

A wide variety of chemical compounds are found in plants, and many of them have been shown to have beneficial effects on appetite, growth and the immune status of fish acting through different mechanisms. Phytochemicals contained in herbs may enhance the innate immune system, possess antimicrobial capabilities and have antioxidant properties that may help to improve the general physiological condition of fish. Many studies have discussed the values of phytochemicals as feed additives. Phytochemicals also act as endocrine modulator that can be applied in aquaculture targeting the production of table fish as well as in ornamental fish production, e.g. carotenoids (carrots, tomatoes, fruits, vegetables), sulfides (garlic, onion), cinnamon, coriander, turmeric.

2. Probiotic Microorganisms

Probiotics have been used in aquaculture to enhance the growth of cultured fish and shrimp. Probiotics is live microbes which is supplemented in feed and it benefits host animal by improving its intestinal microbiota and make a balance between harmful and beneficial microbes. Probiotics or their products have been found useful for health benefits and disease prevention in fish culture system. Probiotics are widely used in aquaculture to improve growth performance, nutrition, decrease diseases and develop immune system.

Gut probiotic

The gut is a fundamental organ system which makes up two equally important functions, i.e., the digestion and host defence. It is very much important to elicit the well-functioning and healthy gut, the dynamic balance of gut ecosystem. A wide range of factors related to diets and infectious disease agents affects this balance and subsequently affect the health status and production. Some bacterial and fungal strains of probiotics can be blended together and can be incorporated with feed pellets or can be encapsulating into live feed or by orally administered to prevent disease and enhance immune system by improving essential microbial flora of the gut.

Water probiotics

water probiotics are applied to reduce organic pollutants and various contaminants in water by directly applying to rearing medium. These improve water quality by converting organic matter to smaller units. Probiotic bacteria such as *Bacillus* sp. can convert organic

matter to CO_2 so that organic effluent can be minimized in aquatic system. Probiotics shows a new dimension in disease resistance and improving water quality in aquaculture industry.

3. Nutraceutical Enzymes

These are enzymes that are derived from plant, animal and microbial sources. Enzymes are an essential part of life, without which animal and human bodies would cease to function optimally. Medical conditions such as blood sugar disorders, obesity, digestive problems and their symptoms eliminated by enzyme supplements in the diet.

B. Non-Traditional Nutraceuticals

These are the artificial foods developed via biotechnology. The bioactive components in food samples are engineered to produce products for wellness of human and animals. They can be two types such as fortified nutraceuticals and recombinant nutraceuticals.

1. Fortified Nutraceuticals

The product which is isolated or purified from the food and sold in medicinal form not associated with food and seems to have a physiological benefit is generally referred as fortified nutraceuticals. A fortified food or a dietary supplement that provides health benefits. While preparing fish feed, the bio-active feed ingredients are mixed with basic nutrients to regulate diversified physiological functions of the fish body in appropriate combination. When food is prepared with scientifically with or without knowledge of how or why it is being used, the food is called "functional food". Functional food provides required amount of vitamins, fats, proteins, carbohydrates to the fish body for health and survival.

Prebiotic

Prebiotics are non-digestible food ingredient that stimulate the growth or activity of beneficial gut commensal bacteria in host thus improves host health. It is reported that a food ingredient which acts as prebiotic such as showing resistance to gastric acidity, hydrolysis by digestive enzyme, fermentation by gastrointestinal microflora and increase the abundance of intestinal bacteria related to health.

2. Recombinant Nutraceuticals

Recombinant nutraceuticals include the making of probiotics and the extraction of bioactive components by enzyme or by fermentation as well as genetic engineering

technology. Also, energy-providing foods, such as bread, alcohol, fermented starch, yoghurt, cheese, vinegar, and others are produced using modern biotechnology.

Mode of action of nutraceuticals

Nutraceuticals function by increasing the supply of important building blocks to the body. The supply of these essential building blocks can be done by two ways by:

(1) reducing signs of the disease as buffering agents for relief.

(2) directly providing health benefits of the individuals.

Benefits of nutraceuticals

There has always been concerns about the benefits of nutraceuticals and the side effects that come along with them. Nutraceuticals have proven to be immunity boosters such as growth enhancement and an increase in the survival rates of the fish under stress. Research has shown that nutraceuticals like flavonoids, green tea, quercetin in onion seems to enhance survival and reduce stress and vitamin E, creatine, turmerine aid in recovering from degenerative diseases. To name a few useful compounds such as antioxidants, fiber, minerals, vitamins, flavonoids are beneficial. Soy foods, flavonoids, green tea extracts have benefits against obesity. Some of the compounds such as green tea, dietary fibers, antioxidants have proven to be antidiabetic also. Some are anticancer agents such as lycopene, soy foods, saponins from spinach, tomato, and potato.

Conclusion

Despite the potential benefits to health and performance as noted in various terrestrial species including human, sufficient dat is not available about the use of nutraceuticals in fish culture. Nutraceuticals that can be used as growth-promoters in fish species and for monosex fish production may provide valid alternatives to synthetic compounds. Comprehensive and coordinated research efforts need to be oriented in every respect to further increase the use of nutraceuticals in fish culture. Such efforts will result in increasing the sustainability of aquaculture system.

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Soil and water quality management for viable brackishwater finfish farming

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A pond with good soil and water quality will produce healthier animals than a pond with poor quality. Poor environmental conditions in ponds bring in a state of stress that is unfavourable for the cultured animals but favourable for the disease-causing agents. Soil and water quality can be maintained within the optimal range by giving importance starting from site selection, suitable pond preparation and culture period. In recent years, the sustainability of brackishwater farming has been questioned because of the various environmental concerns raised. There is a need for maintaining water quality for increased production and ecosystem impact. Understanding pond water and soil characteristics and optimum requirements help to decide the better management practices (BMPs) to be followed in terms of liming, manuring, fertilization, water management etc., for increasing nutrients use efficiency and productivity of the ponds in general and thereby augmenting aquaculture production by reducing the risk of health problems, reduce or mitigate the impacts of farming on the environment. Management strategies for sustainable farming can be done at two levels, one is at the level of the farm which involves proper utilization of resources and inputs and has to be followed by the farmer and the other is at the level of policymakers to integrate aquaculture in the overall development plan of the coastal zone.

1. Water and soil requirements for brackishwater aquaculture

Before initiating aquaculture operation, one should be well acquainted with the nature and properties of water and soil at the production site. Water quality and quantity determine the success or failure of culture operation. Day-to-day management of ponds requires only an estimation of the topping-up rate of the water supply to combat evaporation and seepage losses. An annual water budget should be calculated for a potential farm site so that the supply is adequate for existing and future needs. The optimum ranges of water parameters required for aquaculture are given in Table 1.

A detailed sub-soil survey is essential to ascertain the suitability of the site for pond construction. The sub-surface soils should be tested for compaction and seepage properties, as well as surveying contours. Soil samples from different depths at random points within the site should be analysed for important parameters (Table 2) and heavy metals content. Some soils may have undesirable properties like potential acid sulphate acidity, high organic matter content or excessive porosity. The soil pH ranging between 6.5 and 7.5 is best suited for brackishwater environment for mineralization of organic matter, maximum availability of nutrients and bacterial activities. Soil organic matter is an important index of soil fertility and also helps in the prevention of seepage loss, increases the arability of pond soil bottom and supplies nutrients. The soil rich in $CaCO_3$ content promotes biological productivity as it enhances the breakdown of organic substances by bacteria creating more favourable oxygen and carbon reserves.

Table 1. Water and soil requirements for brackishwater aquaculture

Water parameter	Optimum range
Temperature (°C)	28-32
pH	7.5 – 8.5
Salinity (ppt)	15-25
Transparency (cm)	30-40
Total suspended solids (TSS) (ppm)	<100
Dissolved oxygen (DO) (ppm)	4.0 - 7.0
Total ammonia-N (ppm)	<1.0
Free Ammonia (ppm)	<0.1
Nitrite-N (ppm)	<0.25
Nitrate-N (ppm)	0.2-0.5
Dissolved-P (ppm)	0.10-0.20
Chemical oxygen demand (ppm)	<70
Biochemical oxygen demand (ppm)	<10
Hydrogen sulphide (H_2S) (ppm)	< 0.002

Soil parameter	Optimum Range
pH	6.5-7.5
Organic carbon (%)	1.5-2.0
Available nitrogen (mg/100g)	50-70
Available phosphorus (mg/100g)	4-6
Calcium carbonate (%)	>5.0
Electrical conductivity (dS/m)	>4
Exchangeable acidity (%)	20-35
Depth to sulfidic or sulfuric layer (cm)	50-100
Clay content (%)	18-35
Textural class	Sandy clay, sandy clay loam and clay loam

Even if the site is good with optimum soil and water characteristics, problems may still crop up by the large quantity of inputs like feed and fertilizers, which lead to excessive phytoplankton production, low dissolved oxygen, high ammonia, poor bottom soil condition and other problems. Most of these problems can be avoided by proper management practices during the pond preparation and culture period.

2. Pond preparation

The main objectives of pond preparation are to provide the cultured animals with a clean pond base and appropriate stable water quality. Pond preparation is generally dealt with in two categories viz., newly constructed ponds and existing culture ponds. In newly dug-out ponds, the characteristics of the soil have to be understood first, and soil deficiencies should be identified and treated instead of waiting until poor bottom soil quality develops later. For example, if the soil in a new pond is acidic, it should be limed before the initiation of aquaculture. The pond preparation after harvest before initiating the next crop is entirely different from that of a newly dug-out pond and comprises of removal of waste accumulated during the previous crop by draining and drying of the pond bottom.

2.1 Drying

The pond bottom should be dried for at least 7-10 days for mineralisation of organic matter and release of nutrients. Exposure of the pond bottom to sunlight until it dries and cracks, enhances aeration and favours microbial decomposition of soil organic matter. The optimum moisture content for drying is 20%, but it might vary among soils from different ponds. Pond drying certainly enhances the mineralisation of organic phosphorous but mineralised phosphorus is subjected to available for water column as well as to pond mud.

2.2 Tilling

Tilling bottom soils can enhance drying to increase aeration and accelerate organic matter decomposition and oxidation of reduced compounds. Soil amendments such as agricultural limestone or burnt lime can be mixed into soil by tilling. Accumulation of organic matter and other substances in the surface layer of soil also can be mixed with deeper soils to reduce concentrations of the substances in the surface layer. The pond bottom should not be tilled when they are too wet to support tillage machinery. Ruts caused by machinery will fill with soft sediment and be likely sites for anaerobic conditions. Depth of tillage usually should be 5 to 10 cm, mouldboard plow often called the turning plow, can be used to turn the soil over.

2.3 Liming

The reason for liming aquaculture ponds is to neutralize soil acidity and increase total alkalinity and total hardness concentrations in water. This can enhance the availability of nutrients in the pond water and improve the conditions for the productivity of food organisms

and increase aquatic animal production. Either total alkalinity or soil pH may be used to estimate the liming dose. If both are available but values are not in agreement, use the variable that gives the greatest liming dose. Brackishwater ponds with total alkalinity below 60 mg l^{-1} and any pond with soil pH below 7 usually will benefit from liming. Agricultural limestone will not react with dry soil, so when applied over the bottom of empty ponds, it should be applied while soils are still visibly moist but dry enough to walk on. In ponds with highly acidic soil (pH < 6) liming can increase phosphorus availability by increasing the soil pH. The amount of different lime materials required to raise the pH to7 is given in Table 2.

Table 2. Amount of lime (tons/ha) to raise the soil pH to 7.0.

Soil pH	Quantity of lime material (tons/ha)		
	Dolomite	Agricultural lime	Quick lime
6 to 6.5	5.7 to 2.8	5.5 to 2.8	4.6 to 2.3
5.5 to 6.0	8.5 to 5.7	8.3 to 5.5	6.9 to 4.6
5.0 to 5.5	11.3 to 8.5	11.1 to 8.3	9.2 to 6.9
4.5 to 5.0	14.2 to 11.3	13.9 to 11.1	11.5 to 9.2
4.0 to 4.5	17.0 to 14.2	16.6 to 13.9	13.8 to 11.5

Agricultural limestone will not react with dry soil, hence when applied over the bottom of empty ponds, it should be applied while soils are still visibly moist but dry enough to walk on. Generally, lime is applied after a slight turning over of bottom soil. In soils with chronically low pH, it may be beneficial to apply half the total dosage before slight tilling to neutralize underlying soil layers.

2.4 Fertilisation

Decomposition in organic soils is slow because pH usually is low and the amount of carbon relative to nitrogen (C:N ratio) is high. Urea can be spread over pond bottoms at 200 to 400 kg ha^{-1} at the beginning of the fallow period to accelerate the decomposition of organic soil. Agricultural limestone should not be applied until a few days after urea is applied to prevent a high pH. The rate of application of inorganic fertilizers ranges from 25 to100 kg/ha as a basal dose during pond preparation with a minimum water depth of 10 to 15 cm. When the culture progresses, depending upon the phytoplankton density as exemplified by turbidity of the pond water, the required quantity of the fertilizers may be applied in split doses at short intervals for sustained plankton production.

2.5 Water treatment

Maintenance of good water quality is essential for both the survival and optimum growth of fish. Water treatment is an important step during pond preparation for the maintenance of good water quality at a later stage. Farmers should ensure that only treated water be used in the culture ponds for compensating the evaporation losses.

- Direct use of creek or seawater carries the risk of introducing the virus through aquatic crustaceans. There is a need to eliminate these from the water before use in culture ponds. The use of filter nets of 60-micron mesh/cm^2 in the delivery pipes/ inlet sluice should be strictly followed.
- Water from the source is filtered through filters to prevent the entry of parasites and crustaceans that are carriers of diseases.
- Inorganic turbidity (suspended solids) should be removed by providing sedimentation/ reservoir pond before water to be taken into production ponds.
- Chlorination as a means to sterilise the water is practiced by many farmers. To achieve this enough chlorine should be applied to overcome the chlorine demand of organic matter and other substances in the water. Water should be taken in reservoir ponds and treated with calcium hypochlorite @ 30 ppm. The permissible level of chlorine residuals in treated water for use in grow-out ponds should be less than 0.001 ppm.

3. Management of pond bottom soil during culture period

All aquaculture ponds' soil bottom become covered with sediment, and this sediment can be considered as aquaculture pond soil. In describing various physical, chemical and biological processes occurring in the pond bottom, it is convenient to refer to the bottom deposit as sediment. The sediment-water interface is an intricate system where complex chemical and microbial changes occur and plays important role in brackishwater aquaculture. In the optimum conditions, organic matter present in the soil will be mineralized by using different microorganisms such as autotrophic, heterotrophic microorganisms and it will release the nutrients in the available form.

3.1 Pond treatments to enhance nutrient exchange between soil and water

The two most important nutrients in pond aquaculture are nitrogen and phosphorus because these two nutrients often are present in short supply and limit phytoplankton growth. These two nutrients are added to ponds in fertilizers, manures, and feeds. Fertilizer nitrogen usually is in the form of urea or ammonium, and urea quickly hydrolyses to ammonium in

pond water. Ammonium may be absorbed by phytoplankton, converted to organic nitrogen, and eventually transformed into the nitrogen of fish protein via the food web. Ammonium may be oxidized to nitrate by nitrifying bacteria, and nitrate may be used by phytoplankton or denitrified by anaerobic microorganisms in the sediment. Nitrogen gas formed by denitrification diffuses from sediment to pond water to the atmosphere. Ammonium is in equilibrium with ammonia, and ammonia also can diffuse from pond waters to the atmosphere. A small amount of ammonium may be adsorbed on cation exchange sites in pond bottom soils. Organic nitrogen in plankton and aquatic animal faeces may settle to the bottom to become soil organic nitrogen. Nitrogen in soil organic matter may be mineralized to ammonia and recycled to the pond water, but the rate is slow.

Soils that are near neutral in pH have less capacity to adsorb phosphorus and a greater tendency to release phosphorus than do acidic or alkaline soils. Phosphate is released from iron and aluminum combination when reducing conditions develop from oxygen depletion. A dynamic equilibrium exists between sediment and overlying water so that a small amount of phosphorus is maintained in the solution. Loss of phosphorus from the water is desirable in ponds with large inputs of feed and high levels of aquaculture production because phosphorus from feeds often is a key factor contributing to excessive phytoplankton growth. Phosphorus exchange between soil and water can conceivably be influenced by pond management procedures which influence dissolved oxygen concentrations in the bottom water, disturb the bottom soil surface, suspend soil particles into the water, mix interstitial water into pond water, influence pH, or alter concentrations of iron, aluminum, and calcium.

Fertilization of ponds should be delayed for 1-2 weeks after liming to avoid precipitation of fertilizer phosphorus as calcium phosphate. Alum is sometimes applied to fertilise ponds to remove suspended soil particles and reduce turbidity and also for reducing phosphorus concentrations and phytoplankton abundance. Phosphate fertilizer should be applied 3-4 days after alum treatment to encourage phytoplankton growth and reduce the likelihood of underwater weed infestations developing in the clear water. Elevation of Ca^{2+} concentrations by gypsum treatment can drastically lower phosphate concentrations and retard phytoplankton growth in ponds with naturally low Ca^{2+} concentrations. Application of agricultural limestone or lime [$Ca(OH)_2$, or CaO] might also be effective in precipitating phosphate as calcium phosphate from some ponds. Manual raking of the pond bottom on alternate days accelerated the bacterial activity, improving sediment-water interactions and

nutrient availability suggesting that provision of suitable environmental conditions is as important a requirement as the substrate availability for optimum manure utilization.

3.2 Monitoring of soil parameters during culture period

Monitoring of soil quality can be valuable in fish culture pond management. During culture, the carbonaceous matter, suspended solids, faecal matter and dead plankton etc. also settle at the pond bottom. Major concerns in pond bottom soil management are low soil pH, high soil organic matter, loss of the oxidized layer, and accumulation of soft sediment. Pond managers should still strive to prevent severe soil quality problems from developing. In older ponds with impaired soil quality, problems should be corrected and prevented from recurring. These materials have a combined effect on the environment of the pond bottom. To characterize the soils based on soil type, a pond core sampler (Fig.1) fabricated by the Environment Section of CIBA can be used for the depth-wise collection of cores.

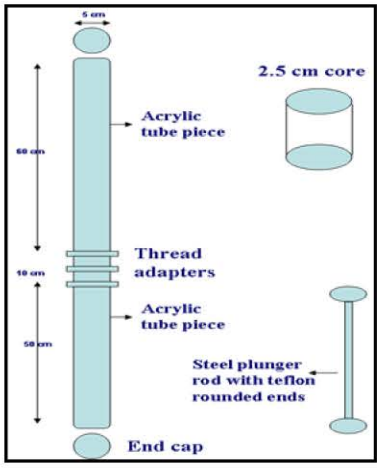

Fig.1. Pond soil core sampler

The low pH of bottom sediment indicates unhygienic condition and needs a regular check-up. The change in the bottom in terms of increasing organic load should be recorded regularly for the management of the pond bottom. The anaerobic condition can be developed in a pond, when the input of organic matter exceeds the supply of oxygen needed for the decomposition of organic matter. This reducing condition can be measured as the redox potential (E_h). E_h indicates whether the water or soil is in reduced (E_h with `-ve' value) or oxidized ($E_h$ with `+' ve value) condition. In anaerobic sediment, some microorganisms decompose organic matter by fermentation reactions that produce alcohols, ketones, aldehydes, and other organic compounds as metabolites. Other anaerobic microorganisms can use oxygen from nitrate, nitrite, iron and manganese oxides, sulfate, and carbon dioxide to decompose organic matter, but they release nitrogen gas, ammonia, ferrous iron,

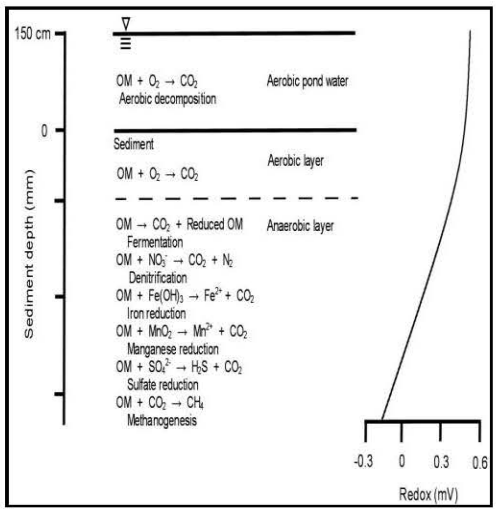

Fig.2 Reactions at the pond bottom soil during aerobic and anaerobic conditions

manganous manganese, hydrogen sulphide, and methane as metabolites (Fig.2). Some of these metabolites hydrogen sulfide, ammonia and nitrite can enter the water and be potentially toxic to fish. The redox potential (E_h) of mud should not exceed -200 mV. The oxidized layer at the sediment surface is highly beneficial and should be maintained throughout the culture period. Ponds should be managed to prevent large accumulations of fresh organic matter at the soil surface, or in the upper few millimeters of soil. Hence, it is extremely important to maintain the oxidized layer at the sediment surface in culture ponds.

4. Water management

Test indicators to ensure that the pond is ready for stocking are secchi disc reading of around 40 cm or less, a stable pH, algal bloom which is brown with a yellowish hue in colour and water temperature above 25^0C.

4.1 Water quality maintenance

The parameters that should be monitored routinely are temperature, pH, salinity, dissolved oxygen and transparency. At the time of stocking fish fry should be acclimated gradually to the salinity of pond water to reduce stress and mortality. The acclimatization rate should not exceed 1 or 2 ppt per hour. Due to the high evaporation rate in summer salinity may increase beyond 40 ppt, which can affect the growth of fish. Sudden fluctuations in the salinity associated with heavy rains result in heavy mortality. Water should be exchanged frequently either by pumps or through the tidal exchange to reduce the salinity variations. Maintenance of salinity of 18 to 35 ppt with variations not exceeding 5 ppt will help in reducing stress on the fish. Water pH can fluctuate between 7.5-9.5 with the accumulation of residual feed, dead algae and excreta over 24 hours with the lowest pH occurring near dawn and the highest pH occurring in the afternoon. The pH should be at an optimum level of 7.5 to 8.5 and should not vary by more than 0.5 in a day. On account of unequal distribution of temperature with a higher temperature near the surface layer and decreasing temperature with depth, thermal stratification can occur resulting in degradation of water quality. The planting of trees on pond dikes to give shade will reduce stratification but at the same time reduce the beneficial effects of wind mixing and restricts solar energy for photosynthesis. The operation of aerators during warm and calm afternoons helps to break thermal stratification by mixing warm surface water with cool sub-surface water. The optimum range of transparency is 25-35 cm. Transparency less than 20 cm indicates that the water is unsuitable for culture and should be changed immediately to flush out excess bloom.

Dissolved oxygen (DO) is the most important and critical water quality parameter because of its direct effect on the feed consumption and metabolism of fish as well as its indirect influence on the water quality. Prolonged exposure to low oxygen content causes low feed consumption which leads to slow growth and the culture organisms become inactive and are susceptible to disease. The concentration of a toxic substance such as unionized/reduced form (NH_3), sulphur (H_2S) and carbon metabolites (methane) increases when a low DO level exists. However, in the presence of an optimum level of oxygen the toxic substances are converted into their oxidized and less harmful forms. The use of aerators results in the mixing of water at surface and bottom and breakdowns DO stratification and also can eliminate black mud formed at the interface of pond water and bottom mud. Water exchange is the best solution to prevent low DO problems in the pond where aeration is not practiced. However, daily water exchange usually does not improve water quality, because routine water exchange can discharge carbon, nitrogen and phosphorous substances from ponds before they can be assimilated. Thus water exchange should only be used when necessary. The number of aerators required is about 1 HP per every 300 kg of biomass. The location of the aerators should be adjusted in such a way the sedimentation occurs at the center of the pond which will aid in its easy removal.

4.2 Metabolites load

The toxicity of ammonia nitrogen is attributed primarily to the un-ionized form and should be less than 0.1 ppm. Un-ionized hydrogen sulphide is toxic to aquatic organisms and the ionic forms, however, have no appreciable toxicity. Any detectable concentration of hydrogen sulphide is considered undesirable. The toxic effect of metabolites can be minimised in several ways. Maintaining a sufficient level of DO facilitates the oxidation of ammonia to harmless nitrate by nitrifying bacteria. Periodic partial removal of cyanobacteria and algal blooms by flushing or scooping out the scum facilitates optimum density and prevents sudden die-off of the bloom.

5. Brackishwater farms discharge water management

Waste production levels from extensive and semi-intensive culture systems are low compared to intensive culture systems. The waste from culture ponds contain mainly suspended solids, comprising of unconsumed feed, faecal matter and plankton, dissolved nutrients phosphorus and nitrogen and metabolites such as ammonia and nitrite. The

overcrowding of farms in certain areas and the limited carrying capacity of the creeks/ estuaries serving such farms has been a matter of concern. The Ministry of Agriculture in its guidelines for sustainable development and management of brackishwater aquaculture has prescribed standards for the water discharged from the aquaculture farms (Table 3). Presently, most of the farms lack a treatment system for treating the discharge water before it is released into the open waters. The discharge water treatment system (DWTS) is mandatory for farms of 5 ha and above as per the guidelines of Coastal Aquaculture Authority (CAA). The establishment of cost-effective DWTS is necessary to bring the brackishwater farms discharge water within the prescribed standards and mitigate any adverse impact on the ecology of the open waters.

Table 3. Standards for discharge water from coastal aquaculture farms in India

Parameters	Final Discharge Point	
	Coastal Marine Waters	Creeks/ estuaries
pH	6.0-8.5	6.0-8.5
Suspended Solids mg/l	100	100
Dissolved Oxygen mg/l	Not < 3.0	0.5
Free Ammonia (as NH_3 – N) mg/l	1.0	0.5
Bio-chemical Oxygen Demand (BOD_5) mg/l	50	20
Chemical Oxygen Demand (COD) mg/l	100	75
Dissolved Phosphate (as P) mg/l	0.4	0.2
Total Nitrogen (as N) mg/l	2.0	2.0

6. Conclusion

The well-designed management practices about soil and water quality should increase efficiency and productivity by reducing the risk of fish health problems, and reducing or mitigating the impacts of farming on the environment. Many of the practices no doubt improve production efficiency in the long run, but a poor cost-benefit ratio may deter farmers from adopting some of them. Regular monitoring of water and soil quality parameters can give an insight into the physical, chemical, and biological environment of the pond ecosystem. Adoption of proper management strategies depending on the site environment characteristics, culture system, the type of management and the needs of the local population will lead to the sustainable development of finfish farming. Government regulations are an important component of management in supporting aquaculture development, maintaining environmental quality, reducing negative environmental impacts, allocating natural resources between competing users and integrating aquaculture into coastal zone management.

Suggested reading

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Recirculatory aquaculture system (RAS) for fish farming

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Recirculating aquaculture systems (RAS) are tank-based systems in which fish can be grown at higher densities under controlled environmental conditions. In a RAS, water flows from a fish rearing tank through a treatment process and returns to the tank, hence the term recirculating aquaculture systems. RAS can be designed to be very environmentally sustainable, using 90-99 percent less water than other aquaculture systems. RAS can reduce the discharge of waste, the need for antibiotics or chemicals used to combat disease and fish and parasite escapes. RAS have been under development for over 30 years, refining techniques and methods to increase production, profit and environmental sustainability. There is a large cost involved in setting up and running a recirculation system and we need to consider a number of factors in designing the system that will fit our needs. This type of aquaculture production system is more commonly used in freshwater environments and can also be used in marine environments. Since failure of any component can cause catastrophic losses within a short period of time, the system must be reliable and constantly monitored. An important component of RAS is the control system which must measure and control all the critical system parameters. Recent developments in control technology and microcomputers may revolutionize the operation and control of RAS. A properly-controlled RAS will also be energy efficient since production can be optimized with respect to the various inputs. In addition, water levels, disruption of electric power, fire, smoke and intrusion of vandals should also be monitored.

Improved Biosecurity

Hatcheries with RAS facility are often fully closed and entirely controlled, making them mostly biosecured - diseases and parasites cannot often get in. Biosecurity means RAS can continuously operate without any chemicals, drugs or antibiotics. Water supply is a regular route of pathogen entry, so RAS water is often first disinfected or the water is obtained from a source that does not contain fish or invertebrates that could be pathogen carriers.

Efficient Management of Water Quality

The most important parameters to be monitored and controlled in an aquaculture system are related to water quality, since they directly affect animal health, feed utilization, growth rates and carrying capacities. The critical water quality parameters that are taken care in RAS are dissolved oxygen, temperature, pH, alkalinity, suspended solids, ammonia, nitrite and carbon dioxide (CO2). These parameters are interrelated in a complex series of physical, biological and chemical reactions. Monitoring and making adjustments in the system to keep the levels of these parameters within acceptable ranges is very important to maintain the viability of the total system. The components that address these parameters can vary from system to system.

A successful water reuse system should consist of tanks, filters, pumps and instrumentation.

Fish Rearing Tanks

The round or octagonal or square design with rounded corners and the arrangement of in- and outlets of water treatment units support the circular water flow. Additional circular water flow and aeration can be enhanced by aqua jets. The circular flow promotes the behavior of fish. Circular tanks are good culture vessels because they provide virtually complete mixing and a uniform culture environment. When properly designed, circular tanks are essentially self-cleaning. This minimizes the labor costs associated with tank cleaning. Typically, water is introduced into a circular tank at the side and is directed tangential to the tank wall. The incoming water imparts its momentum to the mass of water in the tank, generating a circular flow pattern. The water in the tank spins around the center drain, following an inward spiral to the center of the tank. Centrifugal forces and the inward, spiraling flow patterns transport solid wastes to the center drain area where they are removed easily. Once the mass of water in the tank is set into motion, very little energy is required to maintain its velocity. The momentum of the water circling the center drain helps sustain the circular flow. The primary disadvantage of circular tanks is that they do not use space efficiently. A circular tank of a given diameter will have about 21% less bottom culture area than a square tank whose sides are the same length as the diameter of the circular tank. This means that if circular tanks are used there will be 21% loss of potential production in a given amount of space.

Aeration Systems to improve oxygen levels

The most efficient aeration devices move water into contact with the air. The commonly used air stones produce larger air bubbles which rise quickly to the surface and hence the dissolution of oxygen is low. So, the usage of air diffusers are preferred in RAS. These diffusers produce small air bubbles within the tank that rise through the water column. The smaller the bubbles and the deeper the tank, more oxygen is transferred.

Removal of Carbon Dioxide (CO2)

CO_2 is produced through the respiration of fish and microorganisms and will accumulate within recirculating systems if not removed at a rate equal to its production. Elevated CO_2concentrations are not greatly toxic to fish when dissolved oxygen is at saturated levels. For most aquacultured fish, free carbon dioxide concentrations should be maintained at less than 20 mg / L in the tank for good fish growth. CO_2 is usually removed through some form of gas exchange process either by exposing the water to air in a "waterfall" type of environment, or mixing air into the water to remove excess CO2.

Ideal Stocking Density

In evaluating RAS production capabilities, the unit most often used is maximum tank or system stocking density (kg/m^3 or lbs./gallon). However, in terms of production potential, this unit of measure is meaningless. Fish can be held at very high stocking densities while feeding only enough to maintain their basic needs. Underfed fish consume less oxygen and produce less waste. Therefore, the stocking rate of a system ($fish/m^3$) and ultimate maximum fish density (kg / m^3) achieved within a tank should be defined by the maximum feed rate (kg feed / hr or day) that the system can accommodate without wasting feed and still maintain good water quality. This maximum feed rate capacity will be a function of the water treatment system's design, type of fish being grown, and type of feed.

Removal of Solid waste

One of the key problems in RAS is related to the load of suspended solids and in particular to very fine particles. The presence and accumulation of particulate wastes in RAS (faeces, uneaten feed, and bacterial flocs) will negatively impact the water quality by affecting the performance efficiency of the water treatment units. High suspended solids load has many disadvantages:

- ✓ Particulate matter consumes oxygen during biological degradation which will decrease the availability of oxygen for fish in culture
- ✓ The breakdown of organic wastes will increase the Total Ammonia Nitrogen (TAN) concentration in the water affecting nitrification. Small quantities of unionized ammonia can be toxic for epithelial tissues and disturb the elimination of protein metabolites across gills.
- ✓ Solids support the growth of heterotrophic bacteria which can outgrow and compete with nitrifyers. The nitrification process is strongly inhibited by heterotrophic processes when high amounts of organic carbon are present.
- ✓ Particles can potentially clog biofilters and reduce their efficiency
- ✓ Excessive solid loads can cause plugging within aeration columns, screens, and spray nozzles orifices, which could ultimately result in system failure.
- ✓ Suspended solids offer an ideal temporary substrate for facultative pathogens while they try to find a final host. It is also suspected that suspended solids may be involved in bacterial gill disease (BGD) outbreak.

Some type of filters used for the solid wastes are drum filters, bead filters, screen filters and rapid sand filters.

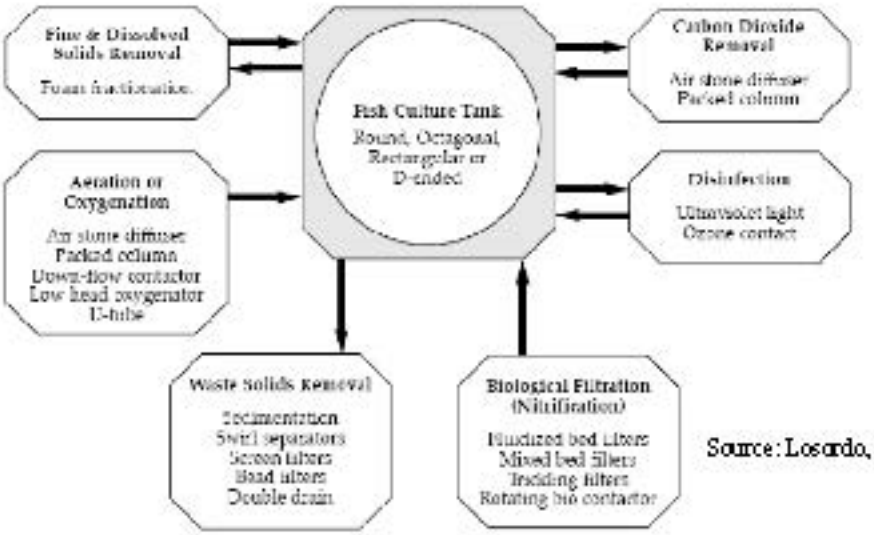
Biofiltration:

In closed aquaculture systems the accumulation of nitrogen compounds, as ammonia and nitrite, has a deleterious impact on water quality and fish growth. The biological filtration (BOD removal and nitrification) is a fundamental water treatment process in every recycling method for the cultivation of aquatic animals. It mainly digests dissolved organic material (heterotrophic bacteria) and oxidizes ammonium-ions via nitrite to nitrate (two-step nitrification) by bacteria like *Nitrosomonas sp.,* and *Nitrobacter sp.* A solid medium is used as substrate for the attachment of the micro flora. Conventional biofilters employ sand or coral gravel as filter media. Modern filters make use of various plastic structures as grids, corrugated sheets, balls, honeycomb-shaped or wide-open blocks. The main goal is to provide a big active surface area for the micro flora settlement. During the last few years moving bed biofilters have received growing attention. These allow to have more specific surface area at the same volume, they need low maintenance due to self-cleaning (no back wash needed). Moving bed reactors are interesting cross between upflow plastic bead filters and fluidized bed reactors. These filters use a plastic media kept in a continous state of movement. The beads are usually buoyant or slightly heavier than water. The specific surface/volume ratio is about 800-1000m^2/m^3. The plastic beads are mixed by hydraulic means driven by air.

Even if nitrate is usually mentioned as the least toxic form in comparison to ammonia and nitrite, high concentrations can reduce immune response and influence osmoregulation in fish. Optimal bacterial growth is the crucial step, otherwise toxic compounds like nitrite, nitrogen or hydrogen sulfide can be formed. The quantity required for denitrification can be calculated on basis of the influent nitrate, nitrite and dissolved oxygen concentrations. The oxidation-reduction potential (ORP) is measured to monitor the denitrification. Sequential removal and reduction of oxygen, nitrate and nitrite result in sequential decrease of ORP in the media.

Protein Skimmer:

Many of the fine suspended solids and dissolved organic solids that build up within intensive recirculation systems cannot be removed with traditional mechanisms. Protein skimmer or Foam fractionation is used to remove and control the build-up of these solids. This process, in which air introduced into the bottom of closed column of water creates foam at the surface of the column, removes dissolved organic compounds by physically adsorbing on the rising bubbles. Fine particulate solids are trapped within the foam at the top of the column, which can be collected and removed. The main factors affected by the operational design of the foam fractionator are bubble size and contact time between the air bubbles and dissolved organic compounds. Foam fractionation is a suitable process in sea water as well as fresh water and the efficiency is increasing with increasing salinities. That is related to the increasing surface tension allowing smaller air bubbles in sea water and there with a higher filter area. Foam fractionation is working very efficiently from salinity of 12ppm and more.



Source: Losordo, et al., 1998

Disinfection of culture water:

Installation of suitable UV sterilizers or ozonisers in the water flow would remove unwanted bacteria, algae and pathogens. The capacity and the flow rate of the UV sterilizer/ ozoniser should be calculated based the on quantity of water to be treated and effectiveness of treatment.

Recirculating Aquaculture System at CMFRI

Drum Filter

Fluidized bed bioreactor

Protein Skimmer

UV Sterilizer

Programmable Logic Controller of the RAS

ICAR - Central Institute of Brackishwater Aquaculture
Kakdwip Research Centre
Training Programme on
"Farming and Seed Production Technology of Brackishwater Fishes" w.e.f. 18 - 23 December, 2021

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"BRACKISHWATER AQUACULTURE FOR FOOD,
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