



Training Manual
on
Farming and Seed Production Technology of Brackishwater Fishes

01 – 07 September, 2022



**ICAR - CENTRAL INSTITUTE OF BRACKISHWATER AQUACULTURE
KAKDWIP RESEARCH CENTRE**

Kakdwip, South 24 Parganas, West Bengal - 743347

**TRAINING MANUAL
ON
FARMING AND SEED PRODUCTION
TECHNOLOGY OF BRACKISHWATER FISHES**

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FOREWORD

The aquaculture sector supports food security, employment opportunities and promotes socio-economic development. Brackish water aquaculture plays a significant role in fetching foreign exchange through export of fish and shrimp. Since its inception, CIBA has been playing an important role in laying the foundations and development of brackishwater aquaculture in India. Over the years, this institute has achieved breakthroughs in seed production of finfishes, indigenous formulated feed development and prophylactic and curative measures of fish and shell fish diseases. Diversifying to other species is considered one of the crucial steps for the sustainable, eco-friendly aquaculture practice. High-value carnivorous fishes like Asian seabass (*Lates calcarifer*), snappers (*Lutjanus spp.*) and herbivorous/omnivorous fishes like grey mullet (*Mugil cephalus*), milkfish (*Chanos chanos*), Brackishwater catfish, *Mystus gulio*, pearlspot (*Etroplus suratensis*), tade mullet (*Liza tade*), and orange chromide (*Etroplus maculatus*) are suitable for farming in the coastal eco-system. Additionally, ICAR-CIBA has also developed different farming methods of brackishwater fishes, viz. Polyculture, multi-tier farming, biofloc-based farming, periphyton-based farming, IMTA, etc. Many of these technologies were successfully commercialized. It is equally important to disseminate the knowledge to the stakeholders to achieve the desired impact of the technologies developed. ICAR-CIBA routinely organizes training programs in Chennai and its regional centers to train personnel in all aspects of brackishwater aquaculture. This year also, Kakdwip Research Center of ICAR-CIBA, Kakdwip, is conducting a 7-day training program on "Farming and seed production technology of brackishwater fishes" during 01 – 07 September 2022 to share the innovative and recent technologies developed by the center among the stakeholders. I am hopeful that the participants would make the best use of this training program and would be confident with gained experience for better aquaculture practices. Also, I believe this special publication would be helpful to the participants in providing appropriate and practical information on various brackishwater aquaculture practices.

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


(Dr. K.P. Jithendran)
DIRECTOR

PREFACE

Brackishwater aquaculture plays a pivotal role in the socio-economic development of the country, and is recognized as a powerful income and employment generator as it causes growth of subsidiary industries besides being a significant foreign exchange earner. Brackishwater sector of the country is endowed with vast and varied natural resources comprising 3.9 million ha of estuaries, 1.2 million ha of potential brackishwater area, 2.54 million ha of salt affected coastal soil and 8.5 million ha of inland saline soils. These natural resources offer immense opportunities for the development of brackishwater aquaculture in the country; which can be substantiated by the fact that the sector has grown at an average annual rate of 10% since 1984. This growth is linked to the farming of single species, tiger shrimp *Penaeus monodon* and more recently, Pacific whiteleg shrimp *Penaeus vannamei*. Outbreak of various viral diseases along with several environmental and social issues in shrimp farming demand diversification of species which have good domestic as well as international markets.

Over the years ICAR-CIBA has generated enormous information on shrimp, fish, crab hatchery and grow-out production, nutrition and feed technology, disease diagnosis and management to address the growing needs of the brackishwater aquaculture sector and provided a platform for interaction with stakeholders. This training program on “Farming and seed production technology of brackishwater fishes” at Kakdwip Research Centre of ICAR-CIBA from 1-7 September, 2022 has been designed to disseminate the scientific information gathered through constant and planned R and D efforts by ICAR-CIBA to stakeholders interested in acquiring apt knowledge and skill for the development of brackishwater sector in the country.

We are extremely thankful to Dr. K.P.Jithendran, Director, ICAR-CIBA for his keen interest and valuable guidance in shaping up this training programme and also for necessary funding support to conduct the training. We are also grateful to Dr. M. Kailasam, Principal Scientist and Section-in- Charge, FCD of ICAR-CIBA for his support in organization and facilitation. We acknowledge the help and cooperation received from colleagues in conducting this training programme. We hope, this CIBA Training Manual Series 2022 No.27 would serve as study material in providing valuable information on brackishwater farming practices and other related aspects relevant for the sustainable development of the sector.



Dr. Prem Kumar



Mrs. Babita Mandal

Place: Kakdwip

Date: 03/09/22

(Training Conveners)



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Brackishwater aquaculture in India-Present status and way forward

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1. Introduction

Aquaculture is the one of the major food production sectors of the world and during the last few decades. In 2020, global aquaculture production touched 87.5 million tonnes (FAO, 2020) where India has contributed 8.6 million tonnes through aquaculture. Aquaculture has important role in the food and nutritional security, economic development and employment opportunities (Kumar and Shivani, 2014; Jayasankar, 2018). Consumption of aquatic food grew from an average of 9.9 kg in the 1960s to a record high of 20.2 kg in 2020 on a per capita basis. India is the second most populous country in the world with a total population of 1.4 billion. The greatest challenge the country faces is to ensure food security of the large 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). Government of India has envisioned enhancing the total fisheries production from 13.7 MMT (2018-19) to 22 MMT in 2024-25. Carps in freshwater and shrimps in brackishwater contribute majorly to aquaculture activity in India. About 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. Under traditional culture system, brackishwater farming in India was 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. 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. History of brackishwater aquaculture in India

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 (mulletts 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, mulletts 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 setback 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. Seed production and Farming of finfishes of brackishwater

3.1 Seed production technology of finfishes

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 Diversified farming systems

3.2.1. Monoculture 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.

3.2.2. 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* (5,000/ha), *Liza tade* (5000/ha), *Mugil cephalus* (2,500/ha), *Scatophagus argus* (2,500/ha), *Mystus gulio* (30,000/ha) and *Penaeus monodon* (2,500/ha), resulted 4,764 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.

3.2.3. 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.

3.2.4. *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.

3.2.5 *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.

3.3 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.

3.4 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 recirculatory aquaculture system (RAS), improved polyculture, biofloc based farming, IMTA, organic farming, periphyton based culture will help to develop sustainable and eco-friendly brackishwater aquaculture.

An Overview of induced breeding techniques of Brackishwater finfishes

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1. 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 due to the non-availability of facilities for the development of captive broodstock and lack of expertise.

2. 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 resilience 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.

3. 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.

4. 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.

4.1. 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.

4.2. 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 transferred to spawning tanks at a density of 1 kg/m³. 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°C. 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°C. 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.

5. 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 200 μg /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 oocyte 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.

An Overview of reproductive endocrinology and induced breeding techniques of Brackishwater finfishes

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1. 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.

2. 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.

2.1 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).

2.2 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).

3. 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). FSH 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 capacitation and spermiation (Miura and Miura, 2003).

4. 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.

4.1 Pituitary gland

The pituitary gland or hypophysis of teleost is located in bony cavity, posterior to the optic chiasma (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 pars distalis (PPD) and pars intermedia (PI). In tetrapods, hypothalamo-pituitary portal system is present whose primary plexus of capillaries is located in the median eminence. The median eminence is 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.

5. Reproductive hormones

5.1 Gonadotropin releasing hormone

The team of Nobel laureates Guillemin and Schally in 1970 characterized hypothalamic hypophysiotropic decapeptide 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 decapeptides, 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).

5.2 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 intra-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).

6. 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 FSH and LH are secreted differently during breeding / reproductive cycle.

6.1 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).

6.2 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).

6.3 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 17 α , 20 β - 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).

6.3.1 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).

6.3.2 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.

6.3.3 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.

6.3.4 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).

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.

7. Induced breeding techniques

The present day concept of the role of pituitary in the reproduction of vertebrates is reported to have originated from the experiments of Aschheim and Zondek in 1927 when they found that pituitary implants accelerated the sexual development of female mice. Three years later, in 1930, Houssay of Argentina performed the first such experiment on a fish.

7.1 Different inducing agents for fish breeding:

7.1.1 Pituitary gland extract:

As in all vertebrates, the activity of the pituitary gland is controlled in large part by a number of neurohormones (neuropeptides, neurotransmitters) that are synthesized by specific neuronal populations in hypothalamus of brain, and reach the pituitary. In all vertebrates, the pituitary is attached to the hypothalamus by a short stalk that, in fish, consists of neurosecretory fibers passing from the brain to the pituitary. These are in fact axons from neurons located in the hypothalamus and sending projections to the pituitary. Pituitary gland extract of mature fish (carp and salmonids) during the reproductive season that contain high amounts of LH, which act directly on gonad and stimulate ovulation and spawning. It has some drawbacks, (a.) the great variability in pituitary LH content (b) the administration of additional hormones present in the pituitary that may adversely affect the physiology of the treated fish, and (c.) the potential for transmission of diseases from donor fish to recipient broodstocks.

7.1.2 Human Chorionic Gonadotropin (HCG):

Purified Human Chorionic Gonadotropin (hCG) directly acts on gonad and stimulate the ovulation and spawning (Zohar and Mylonas, 2001). Recently, an hCG preparation has been approved for commercial utilization in commercial aquaculture (CHORULONTM,

Intervet International bv, The Netherlands). hCG is often given in a single dose, which ranges between 100 and 4000 international units IU per kg body weight. It has a significantly longer half-life in circulation, compared to the gonadotropins of pituitary origin.

7.2 Synthetic hormones:

With the discovery and commercial synthesis of various agonists of GnRH (GnRHa) for human medicine their use for spawning induction therapies in fish increased rapidly, due to their important advantages over LH preparations. In some fishes there is a strong inhibition of basal and GnRH-stimulated release of LH by DA. Therefore, administration of DA antagonists (e.g., domperidone, pimozone, reserpine or metoclopramide) prior to the treatment with GnRHa removes the inhibition on the gonadotrophs and enhances the stimulatory effect of GnRHa on LH release. Many commercial fish breeding hormone is prepared from pharma industries with almost similar chemical formulation. The name of different commercial hormones, chemical composition, and producers contact are given below.

7.2.1 Ovaprim:

Ovaprim is a mixture of the analogue of salmon gonadotropin releasing hormone (sGnRHa) and a dopamine antagonist domperidone (Leelapatra, 1988). Ovaprim, which is marketed by Syndel laboratories Ltd of Canada. sGnRH analogue (Glu-His-Trp-Ser-Tyr-**DArg6**-Trp-Leu-Pro-NH-CH₂-CH₃) has amino acid change in the sixth and tenth position of the native form (Glu-His-Trp-Ser-Tyr-**Gly**-Trp-Leu-Pro-**Gly** NH₂). D-Amino acids, which are mirror image form of the naturally occurring L-forms, have been substituted at position 6 making the molecule much more resistant to degradation in the body and therefore it is long lasting. It contains an analogue of 20 µg of Salmon gonadotropin releasing hormone (sGnPHa) and a dopamine antagonists, domperidone at 10 mg/ml. Ovaprim acts at pituitary level leading to the secretion of endogenous gonadotropin. An additional advantage of domperidone is that it does not cross blood brain barrier in teleosts thereby reducing the probability of detrimental side effect in the injected fish. The dosages of ovaprim that successfully used induced breeding in some selected fish species are 0.2-0.5 ml/Kg of fish (depend on size, sex and maturation stages).

7.2.2 Ovotide

Ovotide is a newly launched ovulating agent developed by an Indian company (Hemmo pharma, Mumbai) which was successfully tested by Central Institute of Fisheries Education (ICAR) Mumbai in several places of India during 1997. Ovotide is a synthetic

preparation containing salmon GnRH analogue and dopamine antagonist. It is composed of 20 µg of salmon GnRH and 10 mg of domperidone/ml. The dosages of ovatide that successfully used induced breeding in some selected fish species are 0.2-0.5 ml/Kg of fish (depend on size, sex and maturation stages).

7.2.3 WOVA FH

Wova FH is manufactured by Biostadt India Limited, Poonam Chambers, 'A' Wing, 6th Floor, Dr.A.B.Road, Worli, Mumbai - 400 018, India. It is available as pills, which contain synthetic Gonadotropin Releasing Hormone Analogue (SGnRH) and dopamine antagonist. Dose of this hormone vary from 0.1 to 0.9 ml/Kg of fish. This dose depends on size, sex species and maturation stages of fish.

7.2.4 OVAFISH

Manufactured by Bhoomiaqua International. F- 304 3rd Floor Durvas CHS Limited Yashwant Viva Township, Near D- Mart, Nalasopara East, Vasai, Thane-401209, Maharashtra, India. Dose vary from 0.2 to 0.8 ml/kg. Composition is s GnRH_a with dopamine antagonist.

7.2.5. Gonopro FH

It is marketed by APC Nutrients PVT. LTD, Minerval House 507, 5th Floor, S.D Road Secundra bad-500003, AndhrapRADESH, India and manufactured by Amrit Pharmaceutical, G-5, MIDC Industrial area, Chikalhana, Aurangabad-431006, Maharastra, India. Dose of this hormone vary from 0.1-0.5 ml/kg of fish. This dose depends on size, sex species and maturation stages of fish. Each ml of this hormone contains 20 µg salmon gonadotropin releasing hormone analog (sGnRH_a) and dopamine antagonist.

7.2.6 Ovopel

This is manufactured by Unic-trade, Hungary. It is available as pellet, which has to be dissolved in 0.9% NaCl solution. One pellet (commercial form) of Ovopel typically contained 18-20 µg mammalian GnRH analogue [D-Ala⁶, Pro⁹- Net]-mGnRH_a and 8-10 mg of metoclopramide (Horváth et al., 1997). Dose ranges from 1-2 pellet/kg of fish. This dose depends on size, sex species and maturation stages of fish.

7.2.7 SPAWNPRO

It is manufactured by PR organics 59-11-4, Kadiyala, Venkaiah Street, Gyatri Nagar, Vijaywada, 520008, India. Each ml. contains Salmon Gonadotropin Releasing Hormone

Analogue 20 µg Domperidone BP 10 mg and Propylene Glycol IPQ.S. The general dose of SPAWN PRO is 0.5 ml per kg body weight of fish. The dose may vary among species and location, female fish 0.3-0.5 ml/kg and male fish 0.1-0.2 ml/kg.

7.2.8 OVASIS:

It is manufactured by Apisa Biotech Extn Private limited, Sai Priya Residency, Saidabad, Hyderabad, Telangana, India. Each ml contains Salmon Gonadotropin Releasing Hormone analogue: 20 mcg Domperidone IP: 10 mg Propylene Glycol IP: qs. Female need 0.3-0.5 ml while male need 0.1 to 0.2 ml/ kg for successful spawning.

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Present status of milkfish (*Chanos chanos*) farming in India

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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 too 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 m² 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. Milkfish hatchery seed production in ICAR-CIBA

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 GnRH α and 17 α -Methyl Testosterone hormones (each 50 μ g/kg). After combined hormone treatment, mature females (mean oocytes diameter 680-700 μ m) and oozing males could be observed under captive conditions. 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. 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.

4. 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.

5. Farming system

5.1. 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/m².

5.2. 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/m³. Offshore cages can be stocked with 40-100 pcs/m³ with a yield from 20-35 kg/m³. Sizes at final harvest typically range from 350 to 500 grams.

5.3. 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.

5.4. 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.

5.5. 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

5.6. 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.

6. 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.

For future reading

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Innovative nursery rearing and farming technologies of candidate brackishwater finfishes of Sundarbans

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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.

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, *Monodactylus argenteus*. Marine seaweeds have been a new area for brackishwater farming in different coastal 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.

2. Nursery rearing technologies of brackishwater fishes

2.1. Nursery rearing of seabass (*Lates calcarifer*) 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./m³. 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./m³ 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.

2.1.2. Nursery rearing of seabass (*Lates calcarifer*) 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 it self can be done wherever possible.

2.1.3. Nursery rearing of seabass (*Lates calcarifer*) in ponds

Nursery ponds can be around 200-500 m² 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./m². 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.

2.1.4. Nursery rearing of seabass (*Lates calcarifer*) 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./m³. 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./m³ 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.

2.2. 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.

2.2.1. Low density fertilization-feeding (FF) system of grey mullet (*Mugil cephalus*)

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.

2.2.2. High density fertilization-periphyton (FP) system of grey mullet (*Mugil cephalus*)

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 nos. @30/-	378900	28290 nos. @20/-	565800
Net return		167233		312251
Benefit-cost ratio (BCR)		1.79		2.23

2.3. 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.

2.3.1. Short duration high density rearing system of milkfish (*Chanos chanos*)

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.

2.3.2. Long duration low density rearing system of milkfish (*Chanos chanos*)

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.

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.

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

Operational cost (OC)	Short duration rearing		Long duration rearing		
	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/-
Net return		494014		158417	288767
Benefit-cost ratio (BCR)		2.39		1.56	2.01

2.4. 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.

2.4.1. Nursery rearing of long whiskers catfish (*Mystus gulio*) 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./m² 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.

2.4.2. Nursery rearing of long whiskers catfish (*Mystus gulio*) in 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.

3. Farming technologies of brackishwater fishes

3.1. Seabass grow-out systems in ponds

3.1.1. 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.

3.1.2. 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 coastal states of India. Successful crops have been demonstrated in Andhra Pradesh, Tamil Nadu and Maharashtra.

3.1.2.1. 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./m³ 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./m³ 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/m³ is possible in the cages, under normal maintenance and production as high as 20-25 kg/m³ is obtained in intensive cage management in the culture of seabass.

3.1.3. 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.

3.2. 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.

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 nos. @30/-	378900 28290 nos. @20/- 565800
Net return		167233 312251
Benefit-cost ratio (BCR)		1.79 2.23

Monoculture of grey mullet depends on availability of suitable seeds as stocking materials. Various on-station and out-station trials have proven that monoculture of grey mullet can be an economically viable farming option provided that ponds are stocked with seeds reared initially in nursery. 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 10000 nos./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.

Polyculture

Polyculture is a farming practice where two or more species of fishes are reared together. The concept of polyculture is based on rearing of two or more compatible aquatic species together resulting 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 nos./ha along with tiger shrimp seeds of 15,000-30,000 nos./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 ton/ha in 6-10 months. The preferred species among fishes are: mullets- *M. cephalus*, *Liza tade* (tade grey mullet), *L. parsia* (goldspot mullet), milkfish- *Chanos chanos*, pearlspot- *Etroplus suratensis* and tiger shrimp- *Penaeus monodon*. In an out-station trial, 3 ponds (1 ha each) were stocked with *M. cephalus* (40-50 g size) as the major species at 10000 nos./ha, *L. tade* (10 g size) at 2000 nos./ha, pearlspot (10 g size) at 1000 nos./ha and *P. monodon* at 4500 nos./ha. Supplementary feeding was provided with floating feed. After 150 days, shrimp with 45 g ABW was harvested and fishes were harvested after 300 days. It resulted in 2.8 ton/ ha production with a net return of Rs. 2.9 lakh/ ha. In another out-station trial, ponds were stocked with *M. cephalus* at 5000 nos./ha, *L. tade* at 5000 nos./ha, *L. parsia* at 10000 nos./ha and *P. monodon* at 40000 nos./ha. After 300 days, total production of 3.6 ton/ ha with a net return of Rs. 5.6 lakh/ ha was achieved.

3.3. 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.



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

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

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.

3.3.1. 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 m²) 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.

3.4. 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.

3.4.1. 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.

3.4.2. 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./m² 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./ m² 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./ m², 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./ m²) in small backyard ponds (300 to 500 m²) will be an ideal practice.

3.4.3. 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./m² with *Oreochromis niloticus* (60 no./m²) and *Rhinomugil corsula* (40 no./m²) 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./m² in polyfarming with *Liza parsia* (stocking density, 0.1 no./m²) and *Penaeus monodon* (stocking density, 15-17.5 no./m²).

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.

3.4.5. 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.

3.5. 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: Mullet- *Mugil cephalus* (striped grey mullet), *Liza tade* (tade grey mullet), *L. parsia* (goldspot mullet), Milkfish- *Chanos chanos*, Pearlscale- *Etroplus suratensis* and Tiger shrimp- *Penaeus monodon*.

3.6. 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.

4. Challenges in advancement of brackishwater fish culture

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

- Poor infrastructure for farming system in different coastal 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 coastal states.
- Poor market intelligence and facilities for marketing of harvested product.
- Poor post-harvest technology.
- Lack of awareness about the new finfishes.

5. 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.

6. 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.

Induced breeding and larval rearing of *Mystus gulio*: cost effective seed production technology

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1. Introduction

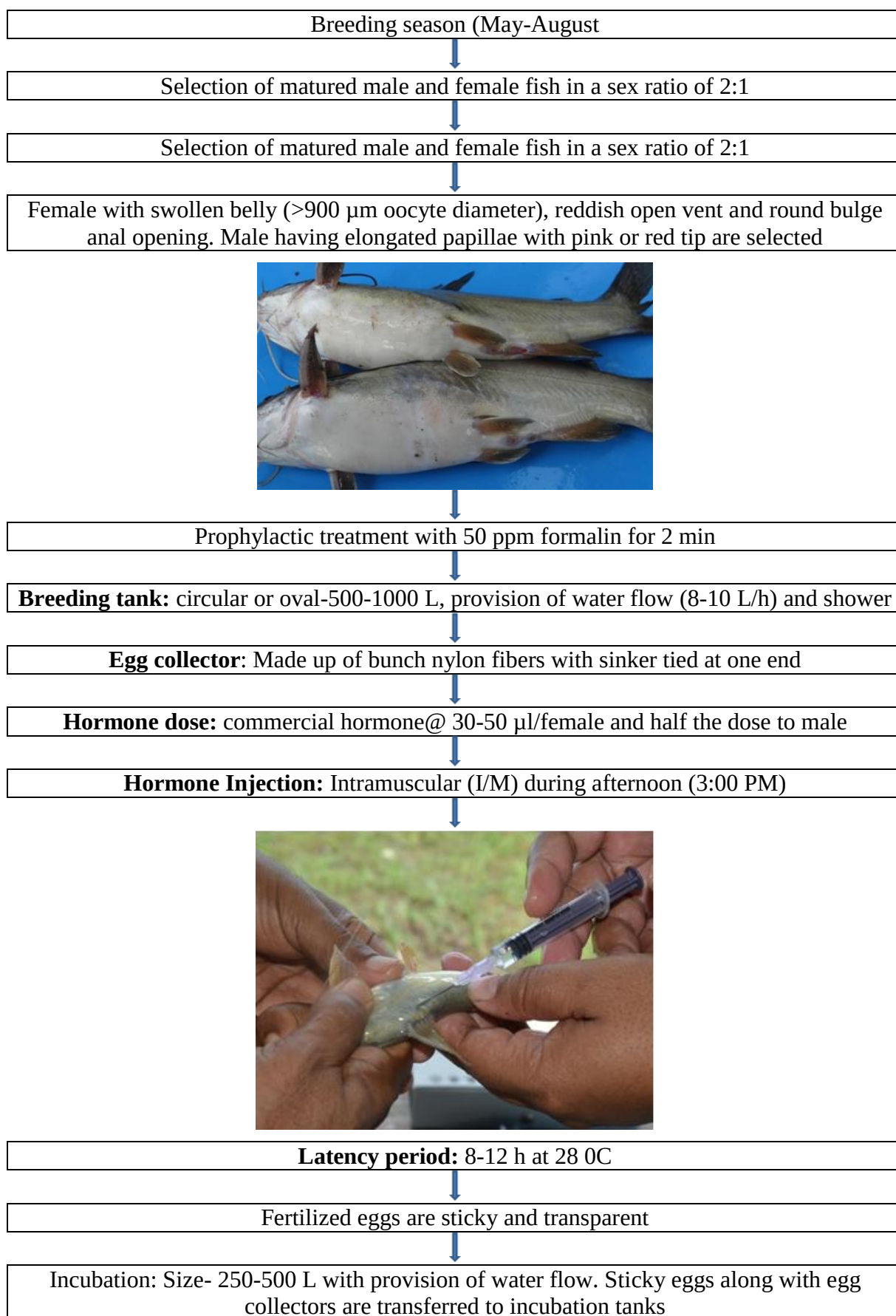
Brackishwater catfish locally known as “nuna tengra”, (*Mystus gulio*) is an important farmed fish of the Sundarban delta. *M. gulio* can be farmed in wide range of salinities ranging from fresh water to brackishwater. This fish easily accept floating and sinking feed. This is an important candidate species for aquaculture diversification because of its hardy nature, delicious taste, excellent nutritional value and high market demand. To meet the seed demand, Kakdwip Research Centre of ICAR-Central Institute of Brackishwater Aquaculture has developed a simplified, improved farmers friendly seed production technology of *M. gulio*, which comprising of captive breeding, larval rearing and nursery.

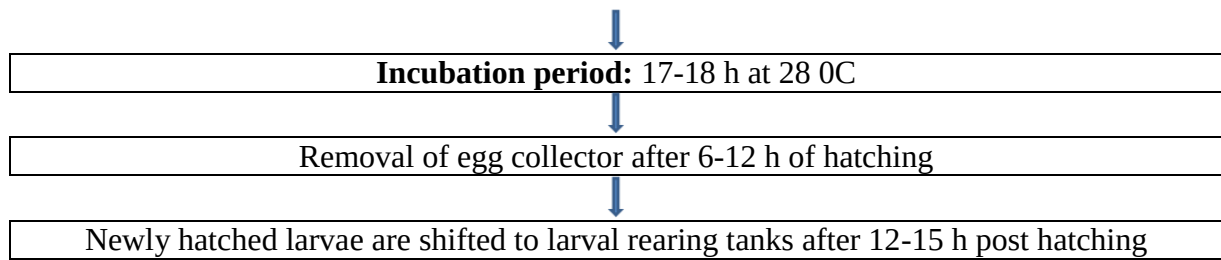
2. Broodstock development

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). 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 m²) at the density of 2/m². During broodstock development fish should be fed with high protein pellet diet (30 % protein, 8% lipid) and/or liver @3% of biomass.



3. Induced breeding





4. Larval rearing

Due to bottom dwelling behavior of larvae, larvae are shifted to larval rearing tanks after 12-15 h post hatching, which reduces handling stress. 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.



Breeding and seed production of brackishwater ornamental fishes

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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.

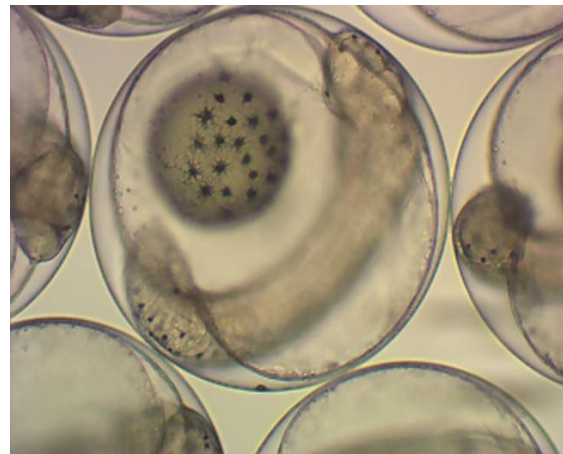
2. Captive breeding of indigenous brackishwater ornamental species at ICAR-CIBA

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 / shell 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 juveniles

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 $\mu\text{g/kg}$ body weight induces the female for spawning. Male fishes are administered with LHRHa @ 200 $\mu\text{g/kg}$ 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.

2.2. Pearlsplit (*Etroplus suratensis*)

Pearlsplit, 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. Pearlsplit 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. Pearlsplit 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 pearlsplit 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. Pearlsplit 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



Eggs on substrate



Wrigglers of pearlspot

2.3. 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.

2.4. 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.

2.5. 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

Current knowledge of on board breeding, nursery rearing and grow out culture of Hilsa (*T. ilisha*) in RAS and pond based systems

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1. Introduction

Tenualosa ilisha (hilsa), the Indian shad belongs to the Clupeidae family, is a commercial fish of the Indo-Pacific region (Bangladesh, India and Myanmar) (Bhaumik 2015; Hossain et al. 2019; Rahman et al. 2018 (Rahman and Wahab 2018 chNGED EVERY WHERE); Sahoo et al. 2016). Hilsa is a migratory fish that mature in seawater and spawn in freshwater river (Hossain et al. 2019; Sahoo et al. 2016). Now a day, the fishing rate of this fish is reduced by a reason of over fishing, river pollution, sedimentation in river, and obstruction in migration (BOBLME 2014; Sahoo et al. 2016; Hossain et al. 2019). Whereas, Bangladesh yearly fishing share as compare to the world total fishing rate is increased from 74.5% (1984–2013) to 86.7% (2010–2015) (Rahman et al. 2018). With the objective to develop conservation strategies and aquaculture of this species, abundant efforts have been made on breeding and larval rearing (Sahoo et al. 2016). Recently, Kumar et al. 2019a, 2020 have studied the artificial breeding, embryonic and larval development, and nursery rearing of hilsa. Scientists have developed the domesticated F1 brood stock of hilsa, which is maturing in captive condition (CIBA newsletter, 2020). De et al. 2020 reared the wild collected seed of hilsa in brackishwater pond enveloped the captive broodstock. Hilsa and Salmon both are anadromous in nature; therefore it is possible that Hilsa migration and physiology may be similar to the Salmon. Important researchable issue to conserve and develop the aquaculture of hilsa are: development of domesticated broodstock, captive maturation through environmental and hormonal manipulation, induced breeding, nursery rearing, grows-out, nutritional requirement and feed development and health management.

2. Taxonomic position:

Hilsa belong to the family of clupeidae, which are characterized by laterally compressed body, keel of scutes along the belly, single dorsal fin, deeply forked tail and ventral fin present far behind the pectoral fins. The Hilsa fish was first described by Russel (1803) at Visakhapatnam shore under the local name of Palash. Hamilton (1822) named the fish as *Clupanodon ilisha*. Regan (1917) nomenclature (*Hilsa ilisha*) remained valid until the current nomenclature as *Tenualosa ilisha*. The caudal fin is as long as head. The body coloration is bluish green on the back and silvery on the flanks. A series of black blotches present along the flanks, which disappear in larger adults (Fig 1). The recent classification and taxonomic position of this species (FAO, 1974) are as followed:

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Clupeiformes

Family: Clupeidae

Subfamily: Alosinae

Genus: *Tenualosa*

Species: *ilisha*

Binomial nomenclature: *Tenualosa ilisha* (Hamilton, 1822)



Fig. 1. Showing captive reared sub-adult hilsa, *Tenualosa ilisha*

3. Migration and life cycle

Hilsa life cycle is identical to American shad, *Alosa sapidissima* (Bhuyan and Talbot 1968) and European shads, *A. alosa* and *A. fallax* (Aprahamian 1982; Bristow 1992). As we know that Salmon is an anadromous fish whereas hilsa is having the nature of both anadromous (migrates up river from the sea to spawn) and amphidromous (migrates from freshwater to the sea, and vice versa, but not necessarily to breed). In general, stocks of *Hilsa* are anadromous in nature (Naidu, 1939), however some stocks do not show migration Blaber et al. 2003; Rahman and Cowx 2006) and some stocks remain always in freshwater stretch of rivers and does not migrate (Hora, 1938; Hora and Nair, 1940).

In India and Bangladesh (Bay of Bengal) two migration periods is described, one from July to October (southwest monsoon migration) and another is from January to March

(winter migration). In monsoon migration, around 90–95% of catches consist of mature fish, while in winter migration the catch is mixed and both mature and immature fish are found (Ahsan et al. 2014). In Myanmar, Baran et al. (2015) identified two periods of upstream migration one in the wet or southwest monsoon season (August to October or even December), and one in the dry season around March. In Myanmar, hilsa are mainly distributed along the Ayeyarwady Delta, which migrates from brackishwater to freshwater areas. This migration takes place through three main routes that is Patheingyi River, the Ayeyarwady River and the Ayeyarwady River delta (Baran et al. 2015). According to the nature of habitat Hilsa is known as an euryhaline and anadromous fish, which attains growth and maturation in the sea and later on migrates to freshwater rivers for spawning (Hossain et al. 2019; Sahoo et al. 2016). Freshwater zones are significant for the Spawning, fertilization, incubation and hatching of the Hilsa.

4. On-board (Artificial) breeding of hilsa

First artificial fertilization of hilsa performed at the lower anicut of the Cauvery River (Coleroon), India by Wilson (1909). Following this, several attempts were made on artificial breeding of hilsa (Raj 1917; Southwell and Prashad 1918; Kulkarni 1950; Jones and Menon 1951; Motwani et al. 1957; Karamchandani 1961; Dixitulu and Chacko 1962; Kumar, et al. 2019a). Dry method is suitable for mass production of larvae (Kumar, et al., 2019a). Most ideal time for artificial breeding of hilsa is evening, when the temperature of water ranges from 23.5°C to 30.0 °C De (1986) and Sen et al. (1990). Hilsa is an oviparous, iteroparous and polygamous (Pillay and Rao, 1963). The diameter of mature/ripe oocytes are 0.70-0.75 mm (Jones and Menon 1951), 0.89 mm (Pillay 1958), 0.76-0.87 mm (De 1980) and 0.769 ± 8.36 mm (Kumar et al. 2019a). Fertilization in hilsa is external, fertilized eggs are buoyed (Kulkarni 1950; Bhaumik and Sharma 2012). The nature of the fertilized eggs of hilsa are greenish-yellow in colour, demersal and make a float with slight disturbance of water. Fertilized eggs are swelled up and look transparent after fifteen mins of post fertilization, unfertilized eggs swell and turned white (De and Sen 1988; Kumar et al. 2019a). The average diameter of fertilized eggs noticed after swelling by De (2014) (2.02mm), De and Sen, (1988) (2.1 to 2.3 mm) Kumar et al. (2019a) (2.03 ± 0.10). Meroblastic type of cleavage is identified in fertilized eggs (De 1914). One hour after fertilization eggs attained morula stage (Kumar et al. 2019). Recent research data described the embryonic and larval development of hilsa (Kumar et al. 2019). According to our observation, blastula and gastrula stage reached 3:30 and 7:30 h after fertilization, respectively. We found the mean incubation period of $21.00 \pm$

1.63 h at 26 ± 0.91 °C of water temperature. Kulkarni 1950 and De and Sen (1988) reported the incubation period of 18-26 h ($27.5-29.5$ °C) and 16-20 h ($24-29$ °C) respectively. Freshly hatched larvae are transparent with single yolk sac few oil globules (Kulkarni 1950 and De and Sen 1988; Kumar et al. 2019a). We have measured the size of the post hatched larvae from 0 to 5 days as 2.50 ± 0.16 , 3.94 ± 0.109 , 4.45 ± 0.22 , 4.71 ± 0.67 , 4.71 ± 0.67 , 5.58 ± 0.84 mm, respectively (Kumar et al. 2019a). Similarly, De and Sen (1988) found the length of larvae from 0 to 5 days as 2.41, 3.75, 3.97, 4.15, 4.42 and 5.12 mm, respectively. The size of the newly hatched larvae ranged from 2.3-3.1 mm (Kulkarni 1950; Motwani et al. 1957; Karamchandani 1961). In larvae, exogenous feeding noticed at 10th or 11th dph and oil globules get utilized on 15th dph (Kumar et al. 2019 and De and Sen 1988). It was observed Hilsa larvae starts exogenous feeding before complete exhaustion of internal food reserve (Kumar et al., 2019, 2020).

5. Nursery rearing and grow-out of hilsa:

Due to its commercial importance at national and international level, it is essentially required to develop the seed production and grow-out technology for hilsa. Recently, Kumar et al., 2019 have studied the detail embryonic and larval development of hilsa. Kumar et al. 2019 studied the ontogeny of hilsa larval development. Efforts are also made to develop larval nursery rearing of hilsa in tank (Chattopadhyay et al. 2019) and pond system (Kumar et al. 2020). Kumar et al. 2020 developed the hilsa larval rearing protocol in pond system. Nursery rearing is carried out in well prepared earthen ponds (100 m^2). Newly hatched 5 days old larvae are stock in well prepared nursery ponds (100 m^2) at the ideal stocking density of approximately 100000 individuals. During nursery, larvae are fed with *Artemia* nauplii (2 g *Artemia* cyst in each treatment) along with powder feed (20 g). At weekly interval fermented GNOC (100 g) is applied in each pond to maintain the productivity of pond water. After 60 days of nursery significantly higher survival of 5% with average size of 4.8 to 5 cm; 2.2 to 2.4 g. Recently scientist attempted to domesticate and develop the F1 stock of hilsa in pond and Recirculatory Aquaculture System (RAS), which showing gonad development (CIBA newsletter, 2018; CIBA Newsletter, 2020). To understand the gonad development of hilsa, macroscopic and microscopic observation of ovary was carried out (ICAR-CIBA annual report, 2020). To understand the growth performance, two months old nursery reared hilsa fingerlings (4.8 to 5 cm; 2.2 to 2.4 g) are stock in two different system viz. earthen pond (1000 m^2) and RAS (8 m^3) in month of May. In both the system, fishes are fed with pelleted feed (38% protein and 8% lipid) @ 5% of biomass. At 15 days interval, lime and GNOC are

applied @ 150 and 50 Kg ha⁻¹, respectively. At fortnight interval regular water quality such as dissolved oxygen (6.5 ppm), pH (8.2) and salinity (6 to 8 ppt) are measured. After 11-months of culture, growth performance of hilsa was visible good in pond system than RAS.



Biosecurity and best management practices in shrimp culture

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1. Introduction

Aquaculture has emerged as one of the major economic activities in the tropical and subtropical regions of the world. To meet the food security needs of the growing human population, food production needs to be improved. Since the land-based resources are depleted over time, now the focus has turned to aquatic farming, as 70% of the earth is covered by a water body. In the recent past, shrimp aquaculture has grown into a multi-billion industry. The growth of the sector is rapid and steady, owing to the latest technological developments, which lead to diversification and intensification of the culture. Intensification of the culture, transboundary movements of cultured species, frozen fishes, and environmental deterioration have led to disease introduction and spread. Frequent Epizootic outbreaks are major limiting factors for shrimp production. The situation has led to huge economic losses to shrimp farmers due to complete crop loss and has an adverse effect on the economy of shrimp farming countries worldwide. Major pathogens of shrimps are as follows, Virus: White spot syndrome virus (WSSV), Yellow head virus, hepatopancreatic parvo-like virus (HPV), monodon baculovirus (MBV), Taura syndrome virus (TSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), Infectious myonecrosis virus IMNV, Laem-sing virus (LSNV), Mourilyan virus (MoV), Shrimp hemocyte iridescent virus (SHIV). Bacteria: *Vibrio parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. campbellii* and *V. harveyi* Protozoan: *Zoothamnium* sp. and *Vorticella* sp. *Enterocytozoon hepatopenaei* (EHP). Disease management is an integral part of aquaculture practices. Disease management in shrimp culture could be achieved through effective diagnosis and treatment, preventive measures, and the adoption of biosecurity measures.

2. Biosecurity

a) Definition

"Biosecurity is defined as a set of standard scientific measures, adopted to exclude pathogens from culture environments and hosts and, more broadly, limit the establishment and spread of pathogens."

b) *Goal*

To protect the culture facility and the surrounding environment from the introduction, exacerbation or spread of pathogens, parasites and pests

c) *Concept*

Biosecurity measures are undertaken to reduce the risk of introducing and spreading disease in shrimp farms. Biosecurity involves a standard set of procedures to prevent the introduction of pathogens or diseases into a population of cultivated shrimp. Such protocols ensure the exclusion of pathogens in culture facilities and prevent the spread of diseases from the facility if infected stocks are present. Implementing effective biosecurity measures in aquaculture facilities prevents health issues due to emerging pathogens and reduces the impact of disease and economic loss due to disease outbreaks. Identification of hazards, assessment of risk and management measures are key steps involved in a biosecurity programme. Aquaculture facilities are always at risk of introducing pathogens and other pests and their exacerbation or spread. Adoption of effective Biosecurity measures can effectively mitigate these risks associated with aquaculture. For the implementation of a biosecurity program, It is essential to understand the pathways and entry points of pathogens and pests into the site. Entry points of pathogens in aquaculture are water, broodstock and stock introduction, stock movement, shared infrastructure, equipment and vessel, farm workers and visitors, wildlife and feed. Biosecurity involves the management of these pathways to prevent the escape or entry of pathogens.

A Biosecurity program involves the management of animals, pathogens and people involved in the programme.

- *Animal*: Stocking healthy animals and optimizing their health and immunity through good husbandry practices.
- *Pathogen*: Preventing, reducing or eliminating pathogens in the system
- *People*: Educating and managing staff and visitors

Better management practices to ensure Biosecurity

Better management practices to ensure Biosecurity in the farm facility include the following activities

- *Fixing barriers*:

Maintenance of physical barriers such as fencing and netting to prevent the entry of other animals such as crabs, birds etc. into the farm facilities. Fixing barriers

prevent predators, wildlife, scavengers and other organisms from entering the farm facility. It helps to prevent the entry of carriers and reservoirs of pathogens.

- *Stocking of healthy seeds:*

Shrimp seeds must be tested before they are brought onto the farm facility and stocked. Shrimp seeds should be tested for OIE-listed diseases through standard diagnostic tools. The process ensures the stocking of disease-free seeds. It is appropriate to stock Specific pathogen-free (SPF) seeds.

- *Effective diagnosis:*

Frequent testing of the stock for the disease is carried out. Regular monitoring and surveillance for the emergence of disease and pests should be implemented throughout the farm. This can predict disease onset and enable to take quick remedial action.

- *Feed management:*

Effective feed management provides adequate nutrition for shrimp and helps to maintain good health. Food-borne disease organisms can be prevented through proper handling and storage of feed. By avoiding over-feeding, water quality could be held at a desirable level. Fixing of check trays and frequent monitoring of feeding is recommended.

- *Quarantine:*

Effective quarantine of available stocks, isolation of infected stocks, maintenance of personal hygiene, control of people, animals and vectors etc., are essential biosecurity measures. Quarantining the infected organisms from other populations is a critical step in the biosecurity programme. Observation of feeding or behavioural changes is highly recommended to assess the fish's health status and take immediate action.

- *Prophylaxis:*

Disease prevention strategies include using immunostimulants, probiotics and bioremediators to improve the culture environment and shrimp health. Using immunostimulants and probiotics improves shrimp health and provides immunity against pathogens.

- *Sanitation and disinfection:*

Frequent sanitation and disinfection of farm facilities allow the reduction of disease-causing organisms present within a given system and prevent or reduce the spread of disease-causing organisms from one system to another. Involves frequent cleaning and removal of farm facilities and all foreign material. Disinfection eliminates most of the pathogenic microorganisms through the application of an appropriate disinfectant. Farm equipment, vehicles and all surfaces within the farm must be included in the cleaning and disinfection process.

- *Water quality management:*

Effective water quality management minimizes the risk of entry of pathogens and pests into the farm. Maintaining good water quality is critical to the health of the fish. Disinfection of water before introduction into the pond is required. During the culture operation, water quality parameters must be maintained at the desired level for the species cultured.

- *Waste management:*

Waste management is a very important step toward attaining Biosecurity on the farm. Aquaculture activities generate waste in the form of liquid and solid materials; before being disposed of, the solid and liquid waste must be treated, disinfected and disposed of to prevent the spread of the pathogen.

- *Documentation and auditing:*

Maintaining the records and frequent auditing of the biosecurity program at regular intervals is key to the successful implementation of biosecurity measures.

3. Conclusion

The appearance of infectious and noninfectious diseases in the shrimp culture system is curtailing shrimp production worldwide. It is high time to develop sustainable, environment-friendly technologies for aquatic health management. Effective diagnostic procedures, incorporating preventive disease management, and strict biosecurity measures result in sustainable, environment-friendly shrimp production and profitability.

Advances in scientific shrimp and mud crab farming: Recent developments

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1. Introduction

Shrimp farming in India emerged from the coastal traditional farming activity where extensive mode of farming was adopted. During the process of this development there had been many pitfalls and hurdles in the form of technology gap and disease issues. Shrimp farming in India took shape into the monoculture form once the farmers started practicing the culture of Tiger shrimp *P. monodon*. However, the shrimp industry has to face major setback in the form of WSSV virus outbreak during the 90's. The outbreak of white spot infection collapsed the Indian shrimp farming industry and majority of the farms became idle. It was at this point the concept of best management practices and scientific shrimp farming was introduced. In the year 2009 to support the shrimp farming sector of the country the government of India approved the import of exotic *P. vannamei* shrimp for culture in the country. The import of *P. vannamei* was a boon to the shrimp farming industry of the country as the SPF broodstock was available. The availability of SPF broodstock and seed is important for the shrimp aquaculture as it can improve productivity by avoiding the introduction of lethal pathogens to the system through the seed. With the introduction of *P. vannamei* the farming sector boomed and today the country produces around 8 lakh tonnes of shrimp compared with the few tonnes it produced during inception. This productivity was achieved not only due to the introduction of the SPF shrimp but also due to the adoption of BMP and scientific shrimp farming practices. The various aspects and recent advances in shrimp farming is discussed in this chapter.

2. Pond preparation and recent developments

The first step of the farming activity starts from the pond preparation. While preparing the pond the major point to be taken care is the soil quality parameters. Since the water quality of the pond is directly related to the soil quality, the parameters like organic carbon, pH, salinity, etc has to be in optimum range. The optimum range of water quality parameters for shrimp farming are as follows, Salinity 5-35ppt, Temperature 28-33°C, Transparency 25-45cm, Dissolved Oxygen 4-7ppm, pH - 7-8.5, Total alkalinity 80-150ppm. At present the

pond preparation methods also includes the pathogen control for WSSV and EHP. If confirmed cases of pathogen attack was there in the pond in the previous crop then the pond preparation methods are modified as follows.

2.1. Pond bottom treatment for WSSV affected pond:

White spot virus stays alive in the pond after 21-25 days even after the draining of the water and in such cases drying the pond for 25-31 days is recommended. After drying, the pond can be filled with water to the depth of 1 foot and bleaching shall be done with 100ppm active chlorine. The pond can be topped up with new water after one week. Further normal procedure for water treatment and fertilization can be adopted.

2.2. Pond bottom treatment after EHP disease:

EHP spores remain in the pond for long time and are tough to kill. Hence the pond bottom needs to be treated with chemicals like Hydrogen peroxide, Sodium Hydroxide and Calcium Oxide. Hydrogen peroxide mixed with water at 1:1 ratio at 150 litres/acre or Sodium Hydroxide at 3000ppm dose sprayed on the bottom or Application of quicklime 3-4 tonnes/acre are the recommended methods to eliminate EHP spores. The selection of the chemical can be done according to the availability and cost effectiveness.

3. Biosecurity measures

One of the key factors of the success of the scientific shrimp farming is due to the adoption of the biosecurity measures. Biosecurity is also a part of adopting the best management practices in shrimp farming. Biosecurity measures are adopted to avoid the entry of pathogens into the farm. Biosecurity starts with the entry of seed into the farm. It is highly recommended to test the shrimp seeds for infection of WSSV and EHP before stocking into the pond which forms the first step of biosecurity. Disinfection of utensils used in the farm can be a source of cross contamination. The machineries and accessories used in shrimp farming has to be disinfected and used properly. Bird fencing has to be provided to scare of the birds as they are known to spread diseases. Crab fencing also has to be provided since crabs are known as carriers of the WSSV disease and it can cause the outbreak of the disease. Apart from this all-unauthorized entry of persons, animals and vehicles has to be prevented to keep the biosecurity status of the farm.

4. Water intake and fertilization

For a successful crop use of disinfected water is highly recommended. Water can be taken from a borewell or creek. It is necessary to filter the water from the creek to remove the unwanted fishes. Water from the creek can be filtered using a 40 and 80 mesh net while

intake of the water. Further, if the turbidity of the intake water is higher than 300NTU, poly aluminium chloride at the dose of 3-5ppm can be administered to settle the clay turbidity. The water can be disinfected with chlorination and the recommend method is the application of bleaching powder at the rate of 15-20ppm. For water fertilization new methods are adopted for better efficiency and plankton production which will enhance the growth and survival of post larvae. Recently the application of inorganic fertilizers is not popular and application of organic juice is getting momentum. Organic juice can be prepared by fermenting the raw rice bran with yeast in pond water. For one acre pond 80kg rice bran, 500 litre pond water and 200 grams yeast are sufficient. All the ingredients can be mixed in a FRP tank and aerated properly. The mixture can be applied to the ponds after 36-48hrs of fermentation.

5. Stocking

Selecting a good quality disease free seed is of prime importance for getting a successful crop. Good quality disease free seeds can be purchased from CAA approved hatcheries. It is also recommended to test the seed prior to stocking for diseases like WSSV and EHP. The seed stocking has to be planned in advance so that the stocking can be done seamlessly. The planned stocking density and number of ponds to be stocked has to be arranged. Acclimatization is the important factor in the seed stocking process and affects heavily the survival of the post larvae. Now it is a trend among the farmers to stock the pond with PL of lower age PL7-8. Farmers prefer to stock lower age PL due to the fact that the transport cost of the lower age PL is lower than higher age PL due to lower number of packed bags. However, stocking of lower age PL can cause problem if not properly done. Acclimatization can be done on the pond side in a FRP tank of suitable capacity. Upon arrival of the seed one or two bags can be opened and the water quality parameters can be checked. If the salinity difference is more than 5ppt the salinity acclimatization has to be done at a rate of 4ppt/hr. During the acclimatization process all the PL can be stocked into the acclimatization tank at a density of 100-200PL/litre. Gradually pond water can be added to the tank and the water quality parameters has to be optimized. The PL can be further stocked into the pond by siphoning using a flexible hose.

6. Feeding and feed management

Feeding and feed management is one of the crucial steps in shrimp farming as it is one of the costly input. Around 60% of the operational cost will be spent for the purchase of feed in shrimp farming. Hence the feeding part has to be practiced with caution. Estimation of feed required is based on the survival and biomass of the animals. In the initial 30 days blind feeding is followed with 100% feeding. Further check tray observation is done to assess the feed intake. Check trays are most important tool for the estimation of the feed requirement.

For the initial feeding any standard feeding chart can be followed. However further feeding shall not take place by blindly following the feeding chart. Check trays has to be placed in suitable places of the pond for monitoring the feeding. Observing the check tray and understanding the feed intake requires practical experience. A sample table for check tray estimation is given below.

DOC	Average body weight (g)	Amount of feed in tray (g/Kg)	Check tray observation time
30	3-3.5	3	2.5 hrs
40	3.5-4	3	2.5 hrs
50	5-6	4	2.5 hrs
60	6-7	4	2 hrs
70	10-13	5	2 hrs
80	13-16	5	2 hrs
90	16-19	6	2 hrs
100	19-20	7	2 hrs
110	20-22	7	1.75 hrs

7. Advances in Mud crab farming

Mud crab is an important candidate species for brackishwater aquaculture in India. The two commercially important brackishwater crab species in India are *Scylla serrata* and *Scylla olivacea*. Out of this two species, the aquaculture activities are concentrated over the green mud crab *Scylla serrata* due to the potential to grow larger and export demand. Live mud crabs demand a niche market in the countries like Singapore, China, Thailand, Japan etc. Small consignments of live crabs are exported on demand basis from the crab export hubs like Chennai, Kolkata, etc. on demand basis to these countries. Unlike other seafood commodities, crabs are exported in live condition individually and the price depends on the individual size. A mud crab of size more than 900g fetches a price of ₹1100-1500 depending upon the demand and season. During the year 2021-22, a total of 4.39 tonnes of crabs (Live fresh or frozen) have been exported to various countries fetching a value of US\$ 35.41million. The higher price per unit of this commodity makes it a sought after species for the brackishwater aquaculture. However, several bottlenecks and technical difficulties in the seed production, nursery and farming side hinders the large-scale aquaculture activities of the species. Nonetheless, small scale growout activities and mud crab fattening can be adopted by the coastal households for livelihood enhancement.

8. Biology and molting

Mud crabs belong to the group crustaceans and possess hard-shelled exoskeleton. Mud crabs (*Scylla serrata*) can grow up to 300mm in carapace width (CW) and, body weight

(BW) of 2.5kg in the wild. Mangrove stretches and estuarine areas are the major habitat of mudcrab. Mud crabs grow in size by a unique process called molting, where the old exoskeleton is removed and new shell is formed. Molting is an important process in the life history of crabs as it is important for the growth and reproduction. The molting process is periodical and follows a pattern as the crabs grows in size. Usually after a molting, the weight gain is almost the double of (>100%) the previous weight, which is an important preferred factor for the culture of the species. In other words, a crab of size 100gm may attain a body weight of 180-230g approximately after one molt. However, there is a catch unlike shrimps, the intermolt period is very long in crabs and may take months to complete a molt cycle. The molting duration also increases as the crab grows in size and larger sizes will take longer intermolt period for next molt cycle.

9. Farming methods

There are two different farming models, which are commonly practiced in the case of crab farming (Crab growout and crab fattening). One method is the conventional crab growout where the juvenile crabs are raised in earthen ponds up to the marketable size. Juvenile crabs of size 50-100gm, which are collected from the wild, is stocked in to ponds at a stocking density of 0.5/m². The farming period is approximately 4-6 months with periodic cull harvesting for removing the bigger crabs. During the farming period, the preferred feed is trash fish at the rate of 10% of the bodyweight of the crab daily. Farming of crabs in open earthen ponds are not so popular and are practiced by very few farmers, due to many issues related to the production and growth. One of the major problem is the survival as the crabs are cannibalistic in nature. The molting process in crabs are not synchronous in nature. Due to this, the recently molted, soft shelled crabs are often attacked by the hard shelled crabs in the ponds which results in lower survival of the crabs. Apart from this, the growth is also not uniform in nature as individual crabs have its own growth pattern. Not all crabs grow bigger in size and due to this, the final harvest will have crabs of different size class, which affects the productivity. These are the major reasons why the crab farming is not done in a large scale and it is normally practiced as ancillary activity.

10. Mud crab fattening

Mud crab fattening is a method of crab farming, which includes the rearing of water crabs for short periods. Water crab is the term used for the recently molted crab where the exoskeleton of the crab is soft or semi hard. Such crabs even if bigger in size will have very low meat content in the body. Water crabs are usually rejected from the export consignment

and fetches low value in the market approximately ₹350-450 irrespective of the individual size (400g->1kg). Water crabs come as a bycatch during crab collection from wild and are set aside by the crab traders for sale to crab farmers/local market. Crab fattening method includes the rearing process of water crabs until the shell gets hardened. Usually the fattening cycle has a duration of 20-35 days depending upon the size and molting stage of the crab. Water crabs are fed with low value trash fish at 10-15% of the bodyweight during the fattening period. During the fattening period, the crabs are periodically tested for the hardening of the shell by gently pressing the abdominal segment of the second walking leg. Once the crabs are hardened the same can be sold at the market for higher price. For example, a water crab of 1kg size that was purchased for ₹340-450 will now fetch around ₹1200-1400 in the market. Crab fattening can be called as a value adding activity since higher returns can be achieved in short duration of time with minimal resources.

The above-discussed factors are the major reasons for the wide acceptance of crab fattening method. Crab fattening is highly recommended activity for the livelihood enhancement for the coastal fisher folk due to the given reasons.

- a. Ready availability of water crabs, as traders are located nearer to the landing centres.
- b. Availability of low value trash fish in higher quantities for cheaper price.
- c. Access to open water bodies for construction of pen, cages, enclosures etc.
- d. Prior knowledge in rearing, handling and management.
- c. Lower initial investment.

11. Site selection

Fattening of mud crabs can be ideally done in open water bodies in pens and floating HDPE fattening boxes. Estuarine areas, bays and lagoons with good quality unpolluted brackishwater is ideal for constructing the crab fattening pens and floating HDPE boxes. Brackishwater ponds with good tidal water influx is also ideal for practicing the mud crab fattening. Area near to drain canals and river mouths where freshwater influx occurs are not ideal as it may carry pollutants and create problems in water quality during the culture period. From our experience, fattening can be done in salinities as low as 3-5ppt without major problems. Farmers who are downstream of brackishwater lakes where salinities are lower can also practice fattening. The major water quality parameters to look for while selecting a crab-fattening site are salinity, pH, alkalinity and dissolved oxygen. Diurnal variation of the DO has to be measured in case of open water bodies as low dissolved oxygen can cause mortality of water crabs. The ideal water quality parameters are given in the table 1.

Table 1: Ideal water quality parameters for mud crab farming activity

Sl.No.	Parameter	Range
1.	Salinity (ppt)	3-35
2.	Temperature (°C)	27-30
3.	Dissolved oxygen (mg/l)	>4.5
4.	Alkalinity (mg/l)	110-150
5.	pH	7.5-8.5

12. Rearing of water crabs

As discussed earlier mud crab fattening can be practiced in pen structure erected in open water bodies or floating HDPE boxes. Floating HDPE boxes are not recommended in areas with wave action as it may damage the floating structure. HDPE boxes are a suitable option in ponds and calm water bodies. Pens made up of plastic coated GI mesh netting erected on casuarina poles can be stocked with water crabs procured from the market. The mesh net has to be driven at least 1 foot down to the bottom of the water body to prevent the crabs escaping from the enclosure. Pens of size 50m² and more are suitable for stocking the mud crabs. The crabs can be stocked at a rate of 0.5-1/m² in the pens and if using the HDPE boxes crabs can be stocked individually. While purchasing the water crabs care has to be taken to select healthy and good quality crabs of adequate size. Mass mortality is often reported in water crabs due to poor health and diseases. It is recommended to buy the local collection of water crabs rather than sourcing from far away locations as they may be sitting out of water for many days. Water crabs that have stayed out of water for many days will be weak but may be live. However, such crabs will die within a short period and we may know about the mortality only during the harvest.

Similarly selecting the size of the water crabs for fattening is also very crucial for the profitability of the activity. For any fattening activity to be profitable water crabs of size, more than 500g is the preferred choice. However, this is a difficult process since the crabs will be provided on weight basis and the crabs have to be carefully sorted for adequate size at the traders place itself. This is very important as 1kg of water crabs comes for ₹350-450 and hard shelled crabs weighing 400-500g will only fetch around ₹450-550. If majority of the crabs selected for rearing are smaller, the profitability will reduce drastically. At the same time, picking only bigger crabs are also difficult as water crabs are not readily available on whatever size is preferred. The availability of water crab is solely dependent on the availability from wild and quantity available will vary according to day and season.

Feeding the crabs has to be done daily with good quality trash fish at 10-15% of the bodyweight. Slightly higher amount of feed may be required in open water pens as other

fishes and organisms entering the pen may consume part of the feed provided. Splitting the ration into two equal parts is recommended to avoid wastage of feed. One feeding can be done early morning and the other part after the dawn. Periodically checking the crabs for hardening of the shell and culling of hardened crabs is a best management practice to increase survival by reducing the cannibalism. If all the factors are good and best management practices are adopted a survival higher than 80% can be expected with better profitability.

13. Grading and selling the crabs

Before harvesting the crabs, it is better to talk with the export trader about the quantity required. Exporters will have a particular demand for a day and more than that will not be taken. If all the crabs are harvested on the same day, it will be difficult to sell all the stock and crabs have to be kept out of water unnecessarily. Keeping the harvested crabs outside water will cause unwanted stress and mortality. Harvesting the crabs in small quantities periodically with prior decided quantities will help in disposing the stock efficiently. The exporter will grade and sort the crabs according to the size and the amount for the product will be calculated. Some of the crabs will be rejected and small quantities of rejected crabs are normal. Crabs without proper hardening, broken legs, low activity, etc. will be rejected and will fetch only lower price. Such stock can be sold on the same day for a lesser price rather than transporting them back to the rearing facility. The pricing of the crabs are done according to the size of the crabs and the method followed is similar between the traders, the details are given in table 2. Farming the crabs can be planned in such a way that the harvest falls during the period from December to February. During this period the demand for the crab is maximum overseas due to Christmas and chinese new year. The price for larger crabs, >950g can go up to ₹1500 during the festival season. Selecting the farming period during this season will be helpful in selling the crabs for a higher price.

Table 2: Grading and pricing of crabs

Sl.No.	Grade	Weight (g)	Price (₹)
1.	Medium	350-499	650-700
2.	Big	500-750	850-900
3.	XL	750-1kg	1300
4.	XXL	>1kg	1500-1800

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 operation. 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.

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

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

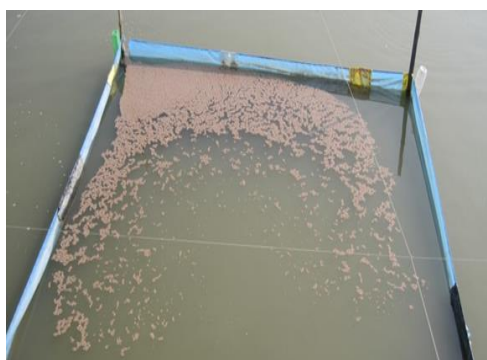


Fig. 3. Feeding in net enclosure

Fig. 4. Feed broadcasting in pond

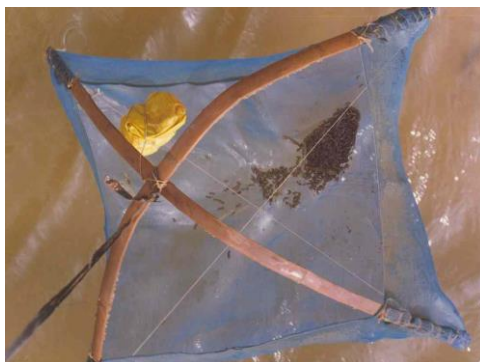


Fig. 5. Feeding in feed tray



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 pereopods. 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
132-138	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	6.1-10.6
21-30	600	11.2-16.6
31-50	700	17.3-30.60
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
1 st month	40	-	-	60	-
2 nd month	40	-	-	30	30
3 rd month	20	20	-	30	30
4 th and 5 th 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 storage 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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Important live food organisms from Sundarbans for fish larval rearing: isolation and mass culture techniques

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1. 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.

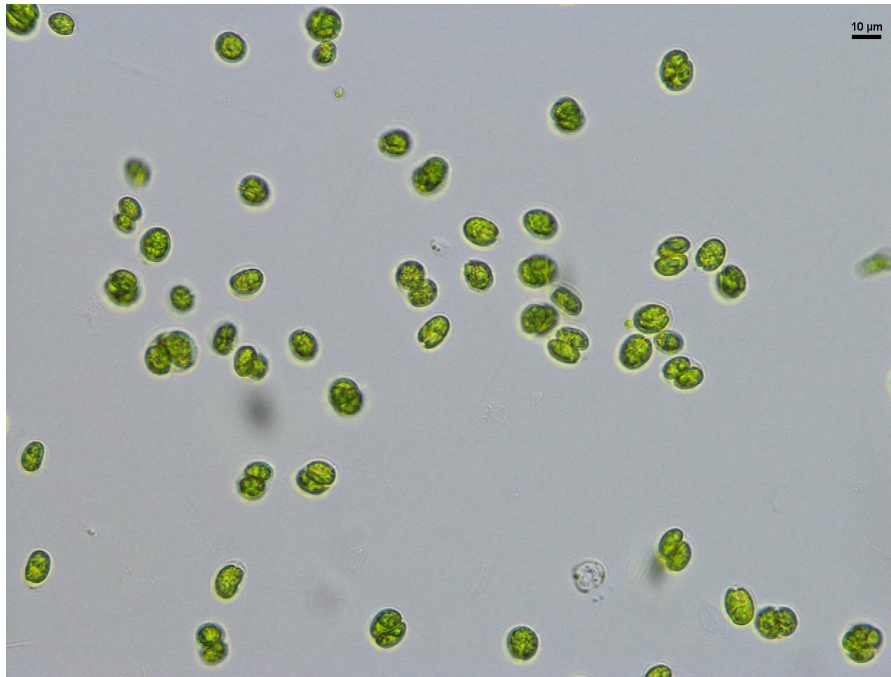
Mainly live feeds consist of phytoplankton and zooplankton grazed upon by economically important fishes. They include different group of organisms like, microalgae, rotifers, artemia and copepods. Importance of live feed is due to several factors as small size, rich essential nutrients, broad spectrum 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.

2. MICRO-ALGAE

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.

2.1. 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.



2.2. 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 medium etc). 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.

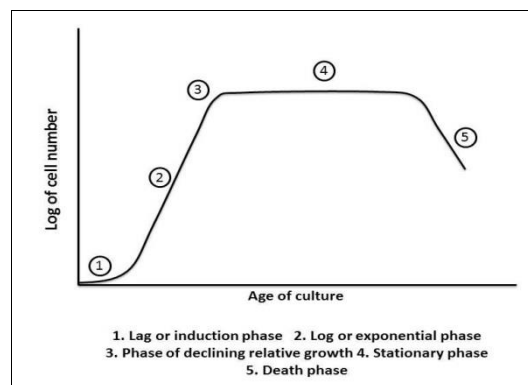


Fig: 1. Different phases in life cycle of microalgae

Indoor culture allows control over illumination (3000-500 lux), temperature ($24 \pm 1^{\circ}\text{C}$), 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 to produce mass cultures of microalgae in open outdoor tanks / ponds for hatchery operations. Different types of microalgae cultures are described in the following section.

2.3. 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).

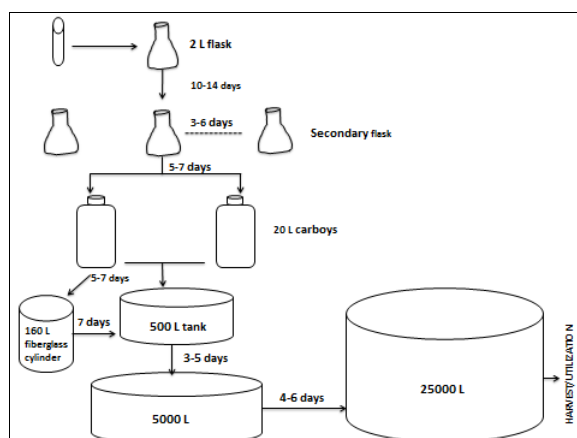


Fig: 2. Diagrammatic representation of batch culture of microalgae

(Source: FAO fisheries technical paper: 361)

2.4. 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.

2.5. 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

2.6. 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.

2.7. 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 oculata* is rich in EPA.

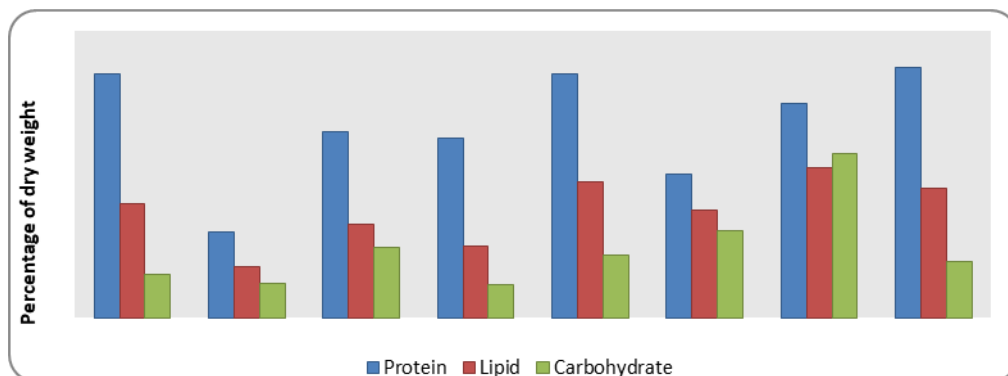


Fig 3. Concentrations of protein, lipid and carbohydrate in some species of micro-algae commonly used in aquaculture

2.8. 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 ₂ O	2.0 g
(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.9 g
CuSO ₄ .5H ₂ O	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.	
(3) Nutrient solution	per litre
FeCl ₃ .6H ₂ O	1.3 g
MnCl ₂ .4H ₂ O	0.36 g
H ₃ BO ₃	33.6 g
EDTA(Disodium salt)	45.0 g
NaH ₂ PO ₄ .2H ₂ O	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) $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	5.65g
(3) Trace elements (chelated)	
Na_2EDTA	4.16 g
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	3.15 g
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.01 g
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.022 g
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.01 g
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.18 g
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	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
$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	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.	

2.9. Fertilizer used for outdoor microalgae culture

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

3. Rotifers

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). Moreover, their small size and slow swimming velocity make them a suitable prey for fish larvae that have just resorbed their yolk sac but cannot yet ingest

the larger *Artemia* nauplii. However, the greatest potential for rotifer culture resides, however, 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 facilitates the inclusion into their body tissues of specific nutrients essential for the larval predators (i.e. through bio encapsulation; see further).

3.1. 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



3.2. Specific requirements to start the mass culture:

For starting rotifer mass culture, algal cell density should be $>10 \times 10^6$ 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.

3.2.1. 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.

3.2.2. 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.

3.3. 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 available in the market for rotifers.

4. Artemia

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 salt pans 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.

5. Copepods

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)

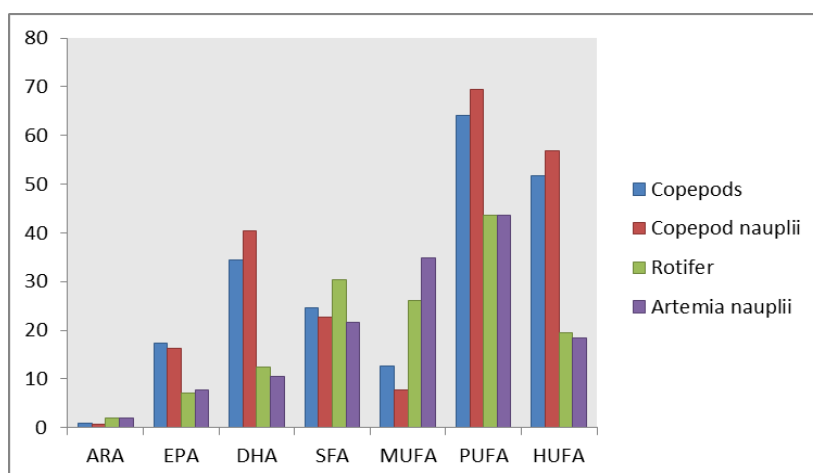


Fig 4. Fatty acid composition of various live feeds (Source: Meeren *et al.*, 2008)

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.

6. 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.

Further readings:

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Disease management of brackishwater finfish in farm and hatchery

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Aquaculture is the fastest growing food-producing sector of the World and among different sectors of aquaculture, the brackishwater aquaculture is the most promising both for livelihood of the farmers and earning of foreign currency through export. 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.

1. Diseases of brackishwater finfishes

1.1. 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.

1.2. 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 clupeioides*, *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.

1.3. 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.

1.4. 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.

1.5. Myxosporidiean diseases

Myxosporidiean are endoparasitic pathogen. The most common genus in this group of parasite is *Myxobolus*. These pathogens primarily infects the gill of the fish. trophozoites of the parasite causes heavy destruction of gill tissue. The infected fish show whirling swimming action. Elimination of the affected fish and disinfection of the environment is the best control method of myxosporidiean parasites. Dip in sodium chloride (3-5%) destroys the spore and other developing stages, but not the cyst.

1.6. 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).

1.7. 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.

1.8. 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, petechial 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.

1.9. 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.

1.10. 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 dinoflagellates. 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 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.

1.11. 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.

1.12. Flukes infections in brackishwater finfishes

The common skin fluke of fishes are *Benedinea* spp. and *Dactylogyrs* 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.

2. 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 m². After 1-2 days of application of lime, potassium permanganate can be applied @ 1 kg per 1000 m².

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/Lit) 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 m²). 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.

3. 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. Mullet 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.

4. Conclusion:

Prevention is always better than cure in case of brackishwater finfish diseases both in case of grow-out culture and hatcheries. In this perspective, it is worthy to mention that the two key aspects of disease management of aquaculture are 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. The management and control of different infections in aquaculture are constant challenge. Disease prevention in brackishwater finfish is accomplished through good water quality management, nutrition and sanitation. Establishment of seed certification system and diagnostic laboratory is important to check the health is important to check the health status of fish brood and seed. Implementation of proper biosecurity measures and setting up quarantine system is necessary to control both introduction and spread of the diseases.

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Fish feed formulation and preparation for fish farming

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1. Introduction

According to the FAO, aquaculture has projected growth to produce nearly 109 million metric tonnes by 2030. Asia contributes about 91% of the world's 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. India is the second largest fish producing country in the world which accounts for 6.56 % of the global production.

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. The total production of shrimp and fish feed in India is close to 27 lakh metric tonnes (MTs) at present which needs to be enhanced in many folds to meet the projected aquaculture growth.

Brackishwater fish species like Asian seabass, mullets, milkfish, pearlspot, *Mystus gulio* need food to get the energy that they require 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 of fish 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 and protein, not only to meet the needs of body maintenance called basal metabolism, but also for growth, reproduction.. 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.

2. Nutritional requirement of different brackishwater fish species

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

2.1. 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.

2.2. 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.

2.3. 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.

2.4. 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 %.

2.5. 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%. The requirement of magnesium (Mg) in 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.

2.6. 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.

3. 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.

3.1. 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.

4. 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, grow-out 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

5. 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-

$$\text{Cost/ kg protein} = 45/0.45 = \text{Rs.100}$$

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

$$\text{Cost/kg protein} = 35/0.40 = \text{Rs.87.50}$$

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 change.

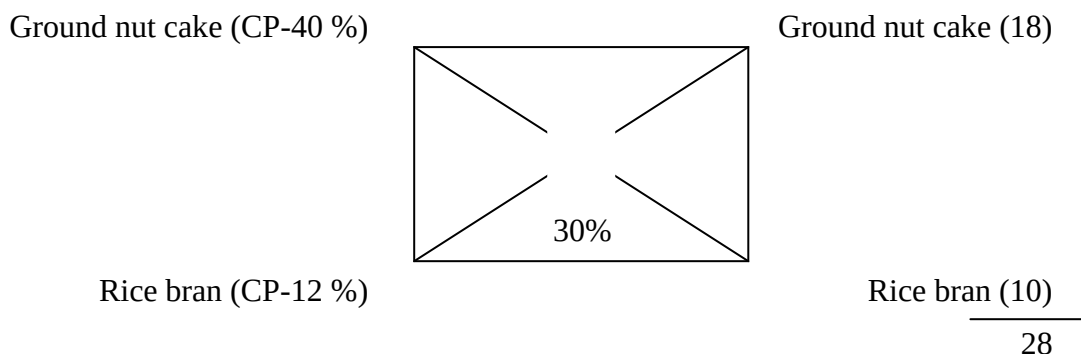
6. Balancing nutrient levels

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.



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

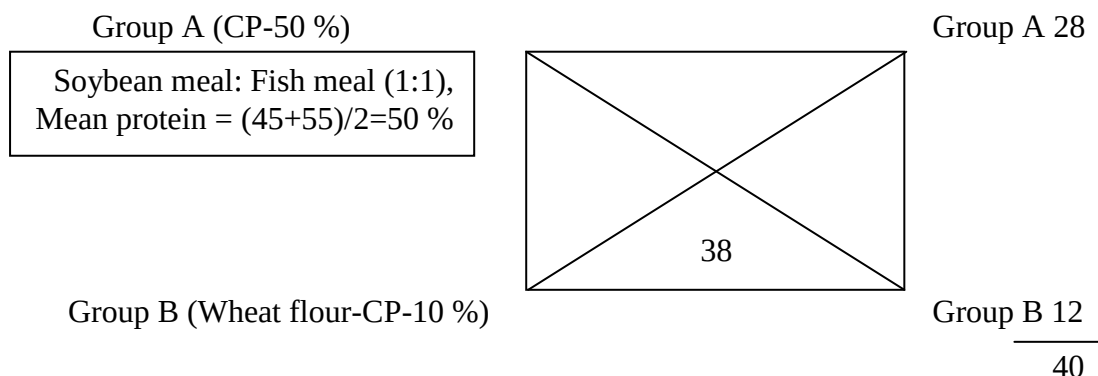
Ground nut cake - $18/28 \times 100 = 64.29 \%$

Rice bran - $10/28 \times 100 = 35.71 \%$

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 - $28/40 \times 100 = 70$ %

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

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

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

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.

7. 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.

8. 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

9. Feed Preparation:

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

Grinding: Grinding of feed ingredients like dry fish, prawn head waste, squid waste, soybean meal etc. individually is the very first step of feed preparation. The coarse grinding can be done with a hammer mill to reduce the particle size. Then all the ingredients is to be powdered in a pulverizer (atta chakki) separately. For further reduction of particle size micro-pulverizer is to be used. Grinding increases the particle numbers and thereby increases the surface area for better action of digestive enzymes with enhanced digestibility and animal performance. Grinding results in better mixing of feed ingredients facilitating in better extrusion and pelleting.

Mixing: Individual feed ingredients is to be weighed as per the formula and to be mixed well in a horizontal or vertical mixer (or hand mixing) except vitamin and mineral mixture. For proper mixing of all the ingredients, a minimum of 15 minutes time is required. With the mixture about 35 litres of water is to be added for a batch of 100 kg and it is to be mixed/homogenized for another 10 minutes.

Steam cooking: The feed mixture is to be loaded in trays and trays are to be kept in steaming chamber for 5-10 minutes. If steaming chamber is not available, the mixture is required to be cooked in a big iron/aluminium container at 60-80 °C temperature for 5-10 minutes. The feed mixture is to be taken out for further cooling.

Incorporation of Vitamin-Mineral mixture: The required quantity of vitamin and mineral mixture is to be added to the steamed and cooled feed mix and to be mixed/homogenized properly into a dough mixer.

Pelletisation: The feed mixture is to be pelletized in a pelletizer fixed with desired size of die and feed pellet is to be collected in aluminium trays.

Drying: The trays loaded with moist feed pellet are to be kept into an electrical drier overnight. The temperature of drier is to be adjusted at 60-70 °C and feed is to be allowed to dry until the moisture content becomes less than 10%. If electrical drier facility is not available, then moist pellets is to be dried properly under Sun. Nowadays solar drier is also available for this purpose.

Checking the quality of feed: Dried pellet feed should be physically examined for visual appearance such as uniformity, colour and smell. The pellet should have surface without cracks. Feed may be sampled and analysed for proximate composition (moisture, crude protein, lipid, crude fibre and ash) . Water stability of the pellet may also be tested after 24 h of feed preparation. For fish feed, the feed must be stable in water for a period of 30-60 minutes.

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. The prepared feed is to be stored in well ventilated rooms away from rodents and insects.

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	Soil parameter	Optimum Range
Temperature ($^{\circ}\text{C}$)	28-32	pH	6.5-7.5
pH	7.5 – 8.5	Organic carbon (%)	1.5-2.0
Salinity (ppt)	15-25	Available nitrogen (mg/100g)	50-70
Transparency (cm)	30-40	Available phosphorus (mg/100g)	4-6
Total suspended solids (TSS) (ppm)	<100	Calcium carbonate (%)	>5.0
Dissolved oxygen (DO) (ppm)	4.0 - 7.0	Electrical conductivity (dS/m)	>4
Total ammonia-N (ppm)	<1.0	Exchangeable acidity (%)	20-35
Free Ammonia (ppm)	<0.1	Depth to sulfidic or sulfuric layer (cm)	50-100
Nitrite-N (ppm)	<0.25	Clay content (%)	18-35
Nitrate-N (ppm)	0.2-0.5	Textural class	Sandy clay, sandy clay loam and clay loam
Dissolved-P (ppm)	0.10-0.20		
Chemical oxygen demand (ppm)	<70		
Biochemical oxygen demand (ppm)	<10		
Hydrogen sulphide (H_2S) (ppm)	< 0.002		

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 to 7 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 to 100 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/cm² 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 [$\text{Ca}(\text{OH})_2$, 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.

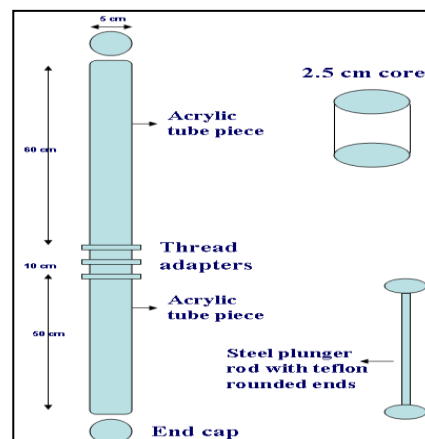


Fig.1. Pond soil core sampler

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, manganous manganese, hydrogen sulphide, and methane as metabolites (Fig.2).

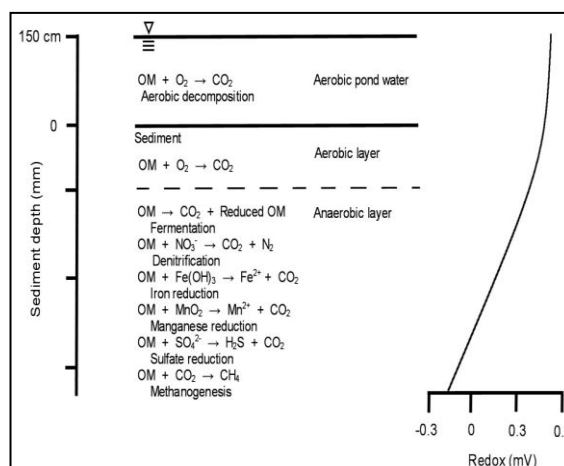


Fig.2 Reactions at the pond bottom soil during aerobic and anaerobic conditions

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°C.

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.

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Emerging diseases of shellfishes and their management

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1. Introduction

Among all the brackishwater crustacean species, the major candidate species for culture in India includes tiger shrimp (*Penaeus monodon*), Indian white shrimp (*P. indicus*), **Pacific white shrimp** (*P. vannamei*), **kuruma shrimp** (*P. japonicas*), **red tail shrimp** (*P. penicillatus*), banana shrimp (*P. merguensis*) and mud crab (*Scylla serrata* and *S. olivacea*). Shrimp Aquaculture has provided a tremendous opportunity for economic and social upliftment of rural communities in the coastal areas of our country. It has been reported that frequent occurrence of infectious disease of bacterial and viral origin have caused severe damage to the aquaculture sector in India and impeded in sustainable development. There are reports of occurrence of emerging diseases in aquaculture. In India farming of *P. monodon* was predominant but due to disease, the production declined. Production was gradually compensated by *P. vannamei*, which was introduced in 2009 through SPF broodstock imported from other countries. Of the total shrimp produced in India, production of *P. vannamei* increased from 3,53,413 in 2014-15 to 8,15,745 MT in 2020-21, whereas black tiger shrimp (*P. monodon*) production reduced from 71,400 MT to 27,616 MT. Mud crab culture in India is limited due to the availability of crab seed for farming. ICAR- Central Institute of Brackishwater Aquaculture, Chennai has standardized the hatchery seed production cycle of *S. serrata* in late 1990s. A package of technology for mud crab culture, as well as production of seed in hatchery available for commercialization, encourages the scope for large scale crab farming in the country. *Scylla serrata* attains a maximum carapace size of 280 mm and 3.5 kg recording even 14–28 g/week weight gain. Scientific crab culture is in demand due to its high value in the live export market and minimum disease risk during the culture period. The interactions between the environment and infectious diseases of cultured shellfish are varied in nature. The natural environment is the source of infectious agents and provides external forces such as temperature and salinity that modify the host – parasite relationship. The aquaculture system is a special environment itself, and one for which management of shellfish health must be learned and practiced. Shellfish infectious disease

agents can be transferred geographically by human activities and also through natural pathways.

The shrimp aquaculture industry has grown rapidly in previous decades due to increasing consumer demand, and it has consequently contributed significantly to the socio-economic development of coastal communities in many developing countries. Production by the shrimp farming industry has steadily increased to approximately 3.6 million t in 2008, accounting for more than 50% of the global shrimp market, with the main production areas being in Southeast Asia, such as China, Thailand, Vietnam, Indonesia, and India, while in the Americas, the major producers are Ecuador, Mexico, and Brazil. Crustacean production totaled 8.4 million t in 2017, representing an average annual increase of 9.92% since 2000, and more than 30 crustacean species were valued at 61.06 billion USD in 2017. However, with the increase in global shrimp aquaculture production, mass mortality caused by frequent disease outbreaks has become a major obstacle for the industry. Worldwide losses from disease in shrimp aquaculture in the last 15 years to 2005 were estimated to be approximately 15 billion USD, 80% of which occurred in Asia. Most shrimp diseases are caused by viral infection, and they have an approximately four times more negative impact than bacterial diseases. In most cases, diseases caused by bacterial pathogens and parasites can be prevented through the proper management of shrimp farms (biosecurity, water quality control, stocking density, aeration, fresh feed, shrimp seed quality, and proper breeding environment), which is in contrast to viral diseases. The occurrence of disease is the reason that existing farmed shrimp species are replaced with other species. The cause of the conversion from *P. monodon* in the 1990s to *P. vannamei* in the 2000s is also closely related to disease occurrence.

2. White Spot Syndrome Disease (WSSD)

Aquaculture practices are responsible for approximately 75% of the world's shrimp production, and the predominant species used are black tiger shrimp, *P. monodon* and white Pacific shrimp, *P. vannamei*. In the past 20 years, shrimp diseases have caused critical economic losses that seriously threaten farming practices, of which white spot syndrome (WSS) is the deadliest viral disease caused by white spot syndrome virus (WSSV). WSSV causes up to 80–100% mortality of infected shrimp within 5–10 days, thus leading to great economic loss; the total economic loss from this disease is estimated to be approximately 8–15 billion USD, and this continues to increase by 1 billion USD (10% of global shrimp production) annually. WSSV infection in shrimp is easily recognized by the characteristic

white spots on the carapace, but WSSV infection does not always show symptoms of white spots and cannot be considered as a reliable indication for the diagnosis of disease, as some bacterial infections, high alkalinity, and stress can also produce similar spots. WSSV infection can cause integumentary dysfunction, resulting in accumulation of calcium salts in the cuticle, resulting in white spot formation.

3. Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV)

Infectious hypodermal and hematopoietic necrosis virus (IHHNV) is a critical viral pathogen of penaeid shrimp, causing serious economic loss to the shrimp aquaculture industry, and has been listed as a reportable crustacean disease pathogen by the World Organisation for Animal Health (OIE) since the year of 1995. IHHNV causes the RDS in juvenile *P. vannamei* and *P. monodon*, which causes stunting in growth, and accounts for 50% of the economic loss in the shrimp industry. IHHNV causes economic damage by reducing the marketability of shrimp due to poor growth, irregular growth, and epidermal malformation during harvest by RDS (cuticular deformities of the rostrum, antennae, thoracic and abdominal areas).

4. Infectious Myonecrosis Virus (IMNV)

Infectious myonecrosis (IMN), also known as Penaeid shrimp myonecrosis virus (PsIMNV), is a major disease caused by the infectious myonecrosis virus (IMNV), which adversely affects the shrimp aquaculture industry. IMNV occurs in *P. vannamei*, its infectious host, and causes infective myonecrosis. The occurrence of this disease is thought to be related to certain types of environmental and physical stress (extreme temperature and salinity, collection by cast-net) and the use of low-quality shrimp feed. Although IMNV can induce an increase in mortality due to an acute infection in *P. vannamei*, the infection is usually detected by observing chronic symptoms in the host rather than a rapid mortality.

5. Yellow Head Virus (YHV)

Yellow head virus (YHV) and gill-associated virus (YHV, GAV) first emerged in the early to mid-1990s and are serious pathogens of the giant tiger shrimp, *P. monodon* farmed in Thailand and Australia, respectively. YHV infection presents typical disease symptoms with yellow coloration of the cephalothorax and gills, but YHV infection can exist for long periods without any signs of disease, such as with the WSSV outbreaks. YHV infection faded the overall body color of the shrimp, and mortality progressed after about 45–60 days of culture, resulting in a cumulative mortality rate of 60–70%. It is reported that after the initial clinical

signs of YHV disease (the development of yellow discoloration of the cephalothorax and gills), 100% mortality occurred within 3–9 days, resulting in rapid damage to shrimp production.

6. Taura Syndrome Virus (TSV)

TSV (Taura syndrome virus) is known as one of the three most critical shrimp viruses alongside WSSV and YHV, as it has seriously damaged the shrimp aquaculture industry worldwide over the past two decades. TSV infection in *P. vannamei* is divided into three stages: acute (7 days after infection with an asymptomatic phase of 2–5 days), transition (lasting 5 days after the acute stage), and chronic (survivors after molting) stages, with a mortality rate of 60–90%. Clinical symptoms of acute TSV infection in farmed *P. vannamei* are characterized by a reddish body color (especially on the tail; uropods, and appendages induced by chromatophore expansion) and irregular black (melanization) spots under the cuticle layer, in addition to anorexia, an erratic swimming behavior, lethargy, soft cuticles, anorexia, flaccid bodies and opaque musculature. The main target organs following TSV infection are the cuticular epithelium of the gills, appendages, hindgut, foregut, and general body cuticle, and the lesion can spread to the underlying subcuticular connective tissue and striated muscle, and even the hematopoietic tissue, antennal gland, testes, and ovaries can become infected.

7. White feces syndrome (WFS)

White feces syndrome (WFS), reported since the last decade, has recently been considered a serious issue was confronting shrimp farming worldwide, especially *Penaeus vannamei* farming in major shrimp farming nations. This disease has been reported from both cultured black tiger shrimp and pacific white shrimp. WFS usually occurs or becomes evident after 30–40 days of culture (DOC), and it may be accompanied by shrimp mortality. Ponds affected with WFS have floating white fecal strings, white/golden brown intestine, reduced feed consumption, growth retardation, and loose shell. The WFS has been reported to be associated with the presence of aggregated transformed microvilli (ATM) structures resembling gregarine worms, vibriosis, Enterocytozoan hepatopenaei, blue-green algae, and fungi.

8. Enterocytozoon hepatopenaei (EHP)

Disease is a major limiting factor in the global production of cultivated shrimp. The microsporidian parasite *Enterocytozoon hepatopenaei* (EHP) was formally characterized in

2009 as a rare infection of the black tiger shrimp *Penaeus monodon*. It remained relatively unstudied until mid-2010, after which infection with EHP became increasingly common in the Pacific whiteleg shrimp *Penaeus vannamei*. EHP infects the hepatopancreas of its host, causing hepatopancreatic microsporidiosis (HPM), a condition that has been associated with slow growth of the host in aquaculture settings. Unlike other infectious disease agents that have caused economic losses in global shrimp aquaculture, EHP has proven more challenging because too little is still known about its environmental reservoirs and modes of transmission during the industrial shrimp production process.

9. Early mortality syndrome (EMS)

Vibriosis is one of the most severe bacterial diseases in shrimp aquaculture, and is caused by the infection of pathogenic and/or opportunistic *Vibrio* bacteria. It can lead to mortality of up to 100 percent and is estimated to cause the shrimp sector US\$ 3 billion in global losses a year. One of the most recent cases of vibrio-related diseases is early mortality syndrome (EMS), which is also known as acute hepatopancreatic necrosis disease (AHPND) and has put a huge dent in the profits from Asian shrimp production in the last decade. Diseases caused by vibrio-related infections may occur more frequently in hatcheries, due to the relatively underdeveloped immune systems of juvenile shrimp.

10. Running mortality syndrome (RMS)

The term running mortality syndrome (RMS) has been used to describe prolonged chronic mortality during a crop. The mortality starts 1-2 months after stocking and become severe during later part of summer crop. This condition has been noticed in farmed *P. vannamei* in India, since 2001. The farmed shrimp in the affected ponds showed different mortality patterns which are result of unusual symptoms with no correlation to any other reported diseases. Reports indicate that some farmers have lost up to four crops, with mortality percentage reaching 50-70% in most of the cases. The exact cause of the disease has not been identified; however, reports indicate the involvement of multiple causes such as covert mortality disease caused by covert mortality nodavirus (CMNV), white muscle syndrome, white gut / faeces syndrome and white patch disease.

11. Health management practices and biosecurity

Managing the health of farmed shrimp species and developing new methods for disease prevention and treatment, preventing the illegal transboundary movement of live

shrimp species, and controlling disease outbreaks through the supply of fresh food worldwide, requires an immense amount of effort.

12. Water quality management

- To reduce risk of disease through contamination, avoid water exchange from outside the farm. Water should be exchanged from reservoirs. Reservoir should be properly treated prior to use.
- Do not exchange water frequently. If necessary, water can be exchanged after 60 DOC but try to minimize water exchange as much as possible.
- Run the aerators for atleast two hours every day.
- Monitoring and management of salinity, pH, dissolved oxygen and microbial load should be done periodically.
- To control water pH within the optimum range of 7.5-8.0, apply lime to increase the pH. High pH results excess plankton bloom from over liming.
- If the pH is higher than 8.3, apply fermented juice to reduce the pH.
- Use agricultural lime after water exchange and after rain. Lime should be mixed with water and spread through out the pond. It acts as a buffering agent for water.
- The water depth at the shallowest part of the pond should be at least 80 cm.

13. Pond bottom management

- Deteriorated condition of the pond bottom directly affects the shrimp. Removal of black and toxic bottom sediments is essential to maintain a healthy environment.
- Check weekly basis, especially at the feeding area for black soil, benthic algae and bad odour. Always take care not to accumulate leftover feed at the pond bottom because later on, it will deteriorate the soil.
- Do water exchange and reduce feeding in areas where the soil is black.

14. Feed management

- Feed management is one of the most important aspects of shrimp aquaculture as the feed accounts for 50 to 60% of the operating cost.
- Feed should be fresh and of good quality to maintain the health and growth of shrimp.

- Determine size and quantity of feed to be applied by following the feed chart. Check the feeding rate, reduce the feeding rate during periods of low DO, plankton crash, rainfall, moulting, extremes of temperature and during disease outbreaks.
- Excessive feeding is more dangerous than underfeeding. So, install feeding trays/ check trays to monitor the feeding. Based on the check tray reduce or increase the quantity of the feed.
- Do regular sampling of shrimps once a week after 45 DOC to determine growth rate and to calculate FCR. Feeding depends on the body weight of the shrimp.
- The method is being perfected based on experience and a standard chart is being prepared.
- Store feed in clean, cool and ventilated area, protected from sunlight.
- It is advisable to switch off the aerators just before feeding and up to 1 hr after feeding.
- First indicator of a serious disease problem: abrupt decline in feed consumption - Virulent infectious diseases and Low dissolved oxygen.

15. Routine Health monitoring

- Daily visual inspection of the animals- Shrimps should be sampled once a week by cast netting and should be checked for their general health conditions like external appearance.
- Note down the visual inspection of the animals like Gill fouling and discoloration, melanized cuticular lesions, abnormal coloration of appendages and chromatophores, soft shelled shrimp, opaque, whitish muscle, deformities and white spots.
- Take observation of pond conditions like shrimp swimming erratically at the surface, dead or moribund shrimp, birds circling overhead and phytoplankton crashes.
- Monitor water quality parameters once a week.
- Probiotics, immunostimulants, bioremediating agents can be working as prophylactic measures in grow out culture.
- Yeast based fermented organic juice can be applied to improve the overall pond microbial balance.
- Monitor for viral, bacterial, fungal and parasitic diseases.
- Diseases may be caused by a single species or a mixture of different pathogens

- Control workers' movement in and across the farm and minimize the number of workers in stocking, harvest, sampling etc.

16. Health and disease management practices during disease outbreak

When viral out-break is suspected need to quarantine the suspected pond. At the same time implement the following during disease outbreak:

- Stop peoples, motorcycles passing across the pond and stop sampling activities
- Check any abnormalities in water and soil condition
- To increase the carrying capacity of unaffected ponds increase number of aerators
- Emergency harvesting, if the mortality rate is increasing rapidly and shrimp are not feeding
- Apply bleaching powder in water to kill shrimps and run the paddle wheel aerators.
- Leave the pond with water for at least 7 days until the dead shrimps become red.
- Do not take paddle wheels out of the pond. Remove dead animals and bury them away from the ponds.
- Bleach pond water for 5 to 7 days before releasing into to the drainage. The pond water should be treated in an effluent treatment system.
- Neighbouring farmers should be kept well informed about shrimp disease problems, emergency harvesting and the time and date of water discharge.
- To avoid the cross contamination during periods of disease outbreak, surrounding farmers should try to avoid water exchange.
- Make sure all people and equipment involved in the quarantine process to follow the biosecurity protocol.
- Better management parctices includes reduced costs and improved Profits, reduced risk to small-scale farmers, increased co-operation and harmony among farmers, better organized farmer groups, reduced disease incidence.

17. Farm Record Maintenance:

- Record keeping is a key component to identify various risks and to rectify these problems at the earliest during the production cycle.
- Records are necessary for identifying water quality problems, monitoring day-to-day activities.

- Record keeping also helps the farmer to learn from past mistakes, thus reducing risk and minimizing costs of production in subsequent crops.
- Record keeping is for accruing the good and bad about the practices adopted.
- To know the net profit or loss.

18. Treatments and use of chemicals

- Antibiotics are very precious and primarily meant for treatment of infections in humans, so avoid using antibiotics
- Antibiotics resistance has become a common phenomenon by misuse of antibiotics.
- Later on, tissue residues may pose a serious challenge in treatment of human infections.
- Antibiotics residues hamper exports resulting in huge loss to farmers.
- Residue in farms affects biogeochemical cycles and productivity.
- Urgent regulation of antibiotic use in aquaculture is needed.

19. Harvesting

- Pre-harvest testing should be done for antibiotic residues.
- Harvesting is done only after the receipt of test report and the report is submitted to the buyer along with the shrimps harvested.
- Harvesting must be avoided during moulting period and agricultural lime can be applied 3-4 days before harvesting.
- Try to harvest in the early morning or evening. Harvesting should be done with a dragnet with minimum delay.
- Harvested shrimps are transferred to ice slurry for chill killing and thereafter shipped to the processing plant in insulated carriers packed in ice.
- The discharged water is routed to the ETS and discharged after ensuring the standards prescribed.

20. Conclusion

Shrimp diseases caused by either Viral or bacterial diminish the production and productivity of the farm and increases the usage of probiotics, and chemicals, thereby impacting the economic condition of the farmers. Stringent biosecurity protocols and right identification of diseases will help reduce the occurrence of spread of diseases from one farm to another. Impacts of health management includes reduced costs and improved Profits,

reduced risk to small-scale farmers, increased co-operation and harmony among farmers, better organized farmer groups, reduced disease incidence, reduced FCR and increased efficiency of resource use. Following strict management and biosecurity measures, higher survivability can be achieved. Biosecurity measures helps to prevent disease introduction and spread, protects your fish, your farm and your investment.

Further readings

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Genome editing and gene transfer techniques in Aquaculture

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1. Introduction

Aquaculture is the fastest growing food-producing sector worldwide, with a 5.3% increase annually from 2001 to 2018 (FAO 2020), and is playing a major and ever-increasing role in meeting human protein needs. It is a critical challenge to produce sufficient animal-based food products with the fast-growing population. In India, the aquaculture industry is an outstanding food-producing sector, providing nutritional security, livelihood support, and employment opportunities to more than 14 million people and contributing to agricultural exports. The sector contributes 1.24% to the total GDP and 7.28% of the agricultural GDP (Handbook on fisheries statistics, 2020). The total fish production of our country is about 14.16 million metric tonnes, among that 10.43mmt contributed by inland fish production. However, different constraints in broodstock development, breeding, seed production, commercial culture of cultivable fishes, etc., still exist in the sector that needs to be addressed.

Advances in genetics will help aquaculture to meet the increasing demand for food in a growing and ever-industrializing human population. Thus, highly productive aquaculture breeds are desirable for producers and consumers (Gjedrem et al., 2012). Technology innovation is imperative to promote food production to meet global demand. Molecular tools and techniques are increasingly used for breeding and seed production of aquaculture species worldwide. Gene editing tools have been successfully used in food fishes by giving particular emphasis to aquaculture species to study gene functions, improve reproductive performance, develop disease-resistant traits, develop phenotypes with quality traits, etc. The newly emerged genetic improvement programs like genome editing showed potential to ease the conventional program in aquaculture. These can introduce favourable changes in the genome and make required genetic traits within a short period. With the high-efficiency gene editing in fishes, we are able to study various gene functions, creating improved traits, disease-resistant fishes, improved reproductive and growth performance, etc.

2. Genome editing:

Genome editing is a technique to precisely and efficiently modify DNA in which a gene of interest is inserted or deleted in the genome of an organism or cells using engineered restriction enzymes, which cut the DNA at its targeted site. Several approaches to genome editing have been developed in recent years with the advancement of technologies, including ZFN (Zinc-finger nucleases), TALEN (Transcription activator-like effector nucleases), and CRISPR/Cas9 (clustered, regularly interspaced short palindromic repeats), which all use an engineered nuclease for targeted DNA cleavage. These newly emerged genome editing methods give precise and powerful gene editing in a diverse range of species, including fish species, with high efficiency (Gaj *et al.*, 2013). All three methods use engineered nucleases to create site-specific double-strand breaks (DSBs) at desired locations in the genome. The induced double-strand breaks are repaired through non-homologous end-joining (NHEJ) or homologous recombination (HR), which are error-prone pathways resulting in the insertion or deletion of nucleotides give rise to the loss of function of a gene.

2.1. CRISPR/Cas9 system

Among the gene editing tools, the CRISPR/Cas9 system has generated much excitement in the scientific community because it is faster, cheaper, more accurate, and more efficient than other existing genome editing methods. CRISPR/Cas9, a tool based on the adaptive immunity mechanism in prokaryotes to eliminate invading genetic material, gained interest in genetic engineering. It consists of two key players that make mutations in the targeted DNA. These are the enzyme Cas9 and a piece of RNA called guide RNA. The Cas9 acts as a molecular scissor that cuts double-stranded DNA at a specific targeted site. So that bits of the sequence can be added or removed. The guide RNA (gRNA) is about 20 bases long pre-designed RNA sequence located within the RNA scaffold. The scaffold part binds to DNA, and the pre-designed guide RNA guides Cas9 nuclease to the targeted genome region, ensuring that the Cas9 enzyme cuts at the right point.

It has been tested in several organisms and found effective in a wide range of species. Based on type II CRISPR/Cas9, co-delivery of endonuclease Cas9 combined with a synthetic guide RNA targeting specific genes into eukaryotic cells can edit the genome by stimulating a double-strand break at the desired site. Then the non-homologous end-joining (NHEJ) process repairs the DSBs, generating insertion/deletion (indel) mutations and enabling existing genes to be edited, deleted, or inserted into new ones. It is a highly efficient and

scalable gene knockout technique that has the potential for applications in zebrafish and other model organisms. It has successfully increased body weight in several fish species, including zebrafish, medaka, channel catfish, *Pagrus major*, *Paralichthys olivaceus*, and common carp (Zhong et al., 2016; Khalil et al., 2017; Kishimoto et al., 2018; Kim et al., 2019). Gene-editing using CRISPR/Cas9 allows rapid body weight increases in shorter periods than traditional selection. Furthermore, superior growth results can be achieved by combining multiple genetic enhancement strategies.

2.2. Applications of CRISPR-Cas9 in Aquaculture

Fishes form the largest group of vertebrates and are important animal models for research, the aquaculture industry, and human diseases. Genome editing technologies use different fish species for basic functional studies of genes, disease modeling, and aquaculture. The most commonly used fishes as the model organism are zebrafish (Bill *et al.*, 2009) and medaka (Aller *et al.*, 2013). The model species have played essential roles in testing new genome editing protocols because of the biological advantages of fish models. Many genes have been disrupted or modified in fish species for functional studies, especially those involved in reproduction. Gene editing tools have also been successfully used in food fishes by giving special emphasis to aquaculture species to study gene functions, improve reproductive performance, develop disease-resistant traits, develop phenotypes with quality traits, etc. Yellow catfish *Pelteobagrus fulvidraco* is one of China's most important freshwater aquaculture species. However, its small size and low fillet yield limit its value. Dong et al. (2011) successfully disrupted the *mstn* gene using zinc-finger nucleases (ZFNs) to create a strain of yellow catfish with heightened muscle mass. They produced two yellow catfish founders carrying a mutated *mstn* gene in their germ cells.

3. Gene transfer methods and their use in aquaculture

Gene transfer technology provides a novel means of altering the genotype of animals and fish to a greater extent than can be achieved by conventional genetic selection procedures. It offers great potential for improving selected genetic traits of agricultural significance in farmed animals. Genetic engineering in aquaculture has the efficiency to enhance production by producing beneficial traits such as enhanced growth, disease resistance, adaptation to extreme environments, etc., by altering the genome of targeted fishes. Growth enhancement in fish using genetic engineering helps increase the bodyweight of food fishes and reduces the culture period, thereby being more effective than traditional

breeding techniques. Although traditional breeding is the most extensively used strategy in crop improvement, it is labour expensive, and it often takes several years to go from the early phases of screening phenotypes and genotypes to the first crosses into commercial varieties. Gene editing and transgenics could produce genetically modified crops with beneficial traits within a short period. Genetically modified (GM) crops with valuable traits are produced by transferring genes (transgenes) of known function into crop varieties. These techniques have the efficiency to combat the growing food demand.

In the early 1980's, with the advancement of techniques in molecular cloning and embryonic micro-manipulation, recombinant genes could be constructed and transferred into the host animals. It has greatly impacted modern biology and biotechnology (Powers et al. 1998). Gene transfer experiments were conducted in model fishes Zebrafish (*Danio rerio*), and Medaka (*Oryzias latipes*) and in food fishes such as Carp (*Cyprinus sp.*), Tilapia (*Oreochromis sp.*), Salmon (*Salmo sp.*, *Oncorhynchus sp.*) and Channel catfish (*Ictalurus punctatus*) Goldfish (*Carassius auratus*). There are different methods to transfer recombinant genes to the host animals.

4. Electroporation

Electroporation is a mechanical method to introduce foreign DNA into a host cell using high-voltage electric shocks. Using high-voltage electric shocks to introduce DNA into cells is a procedure that is gaining popularity for standard gene transfer and allows the generation of genetically modified mice. It can be used with most cell types, yields a high frequency of stable transformation and transient gene expression, and, because it requires fewer steps, can be easier than alternate techniques. Wong and Neumann first demonstrated this method in 1982 to study gene transfer in mouse cells. It is now a widely used method for introducing a transgene into bacteria, fungus, plant, and animal cells. It involves using a large electric pulse that temporarily disturbs the phospholipid bilayer, allowing the passage of molecules such as DNA.

The basis of electroporation is the relatively weak hydrophobic/hydrophilic interaction of the phospholipids bilayer and the ability to reassemble after disturbance spontaneously. Electroporation uses short electric pulses to produce temporary pores on the surface of the target cells through which the transgene is introduced into the cytoplasm, where it is then delivered to the nucleus by the cellular machinery. Electroporation has been

shown to be the most effective means of gene transfer in fish since a large number of fertilized eggs can be treated in a short time by this method.

The disadvantage of the electroporation method is that a tough chorion layer around the fish eggs reduces efficiency; removing the chorion is a tedious procedure and introduces additional stress on newly fertilized eggs. In traditional electroporation methods, either transfer levels have been low with few studies showing germ-line transmission and expression, or the device used is no longer commercially available.

5. Microinjection

Microinjection is a widely used method for gene transfection in mammals. It is the process of transferring genetic materials into a living cell using glass micropipettes or metal microinjection needles. It involves delivering foreign DNA into a living cell (e.g., a cell, egg, oocyte, embryos of animals) through a fine glass micropipette. Glass micropipettes of various sizes with diameters ranging from 0.1 to 10 μm . DNA or RNA is injected directly into the cell's nucleus. Microinjection has been successfully used with large frog eggs, mammalian cells, embryos, plants, and tissues.

Microinjection is a method to introduce DNA into either cytoplasm or nucleus by using a glass needles for injection, a micromanipulator to precise the positioning of the micropipette, and a micro-injector. Using hydrostatic pressure the fluid containing the insert or genetic material deliver into the cell nucleus. Injections are carried out under direct visual control using a microscope. A holding pipette is placed in the microscope's field of view that sucks and holds a target cell at the tip. The small tip diameters of these micropipettes, combined with the high precision of the micromanipulator, allow accurate and precise DNA delivery.

The microinjection method has been successfully used in producing transgenic fish and is a commonly used technique due to its simplicity and reliability. This method ensures higher survival rates for injected fish embryos than the electroporation method. It is the most established method for gene transfer in fish and allows delivery of the transgene directly into the nucleus.

The disadvantage of this method is that, it is a time-consuming and labour intensive procedure for fishes that produce a large number of eggs and also limited by the physiology of fish eggs. The nuclei of fish eggs are difficult to visualize and the chorion hardens soon after fertilization, therefore transgenes are usually injected into the egg cytoplasm.

6. Conclusion

Genetic engineering has great potential in the aquaculture industry. With this high-efficiency technology, we are entering a new era of adopting powerful technologies to study various gene functions and improve their traits. It has the efficiency to produce superior quality strains by introducing desirable genetic traits into fishes. These traits include faster growth rates, improved feed conversion efficiency, disease resistance, abiotic stress tolerance, etc. These are the potential applications of gene transfer technology in aquaculture. This approach may pave the way for growth-enhanced fishes in the aquaculture industry to enhance productivity.

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