

Customized Training in Mariculture for Maldivian Officials

Course Manual



Central Marine Fisheries Research Institute
(Indian Council of Agricultural Research)
Post Box No.1603, Ernakulam North P.O.
Cochin- 682 018 Kerala, India

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The Commonwealth Secretariat
Marlborough House Pall Mall, London SW1Y 5HX, United Kingdom



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18 November- 14 December 2013



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**Customized Training in Mariculture
for Maldivian Officials**

Published by

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PREFACE

Aquaculture is a global production technology, with more than 180 countries reporting some level of production and Asia accounts for the maximum. Though India is not a leading producer in true mariculture we are second in aquaculture production after China. Coastal aquaculture of shrimp has a major role in aquaculture production and export in India. Even though there is vast scope, recently only India has taken up mariculture technologies to the stake holder level. Due to the success achieved mariculture, it has been identified as a potential source of production enhancement for high valued species like lobster, seabass, cobia and pompano for which the capture fishery is negligible.

CMFRI is the premier marine fisheries research institute in India and has trained persons of different categories right from students in the post-graduate and doctoral levels, researchers in different projects of the institute, aquafarmers, entrepreneurs, government officials, teachers, extension personnel and fishermen, in Mariculture and related areas.

The Course Manual being released on this occasion contains the lecture notes presented by the resource persons of CMFRI, NBFGR and CIBA. I have great pleasure to record my gratitude to all the committee members for their dedicated involvement. Dr. G. Syda Rao, Former Director, CMFRI, was instrumental in identifying me for coordinating the training and I thank him for the great opportunity. I thank Dr. A. Gopalakrishnan, Director CMFRI who has been contributing immensely for the successful conduct of the training. I thank him profusely for the valuable suggestions and guidance all through. I thank Dr. G. Gopakumar, Head, Mariculture Division for his whole hearted involvement and arrangements at Mandapam Regional Centre of CMFRI. Dr. P.C. Thomas, In Charge HRD Cell, CMFRI also was very supportive. Dr. Bobby Ignatius, Principal Scientist was with me all through the process and I specially thank him. Other scientists of Mariculture Division at Cochin, Technical staff, research scholars, supporting staff and contractual staff also supported us in organising the training.

I thank all the resource persons who have contributed material for the Course Manual in time. All Heads of Divisions at CMFRI also supported us in this endeavour. I thank the entire Administration and Accounts staff of CMFRI for being such wonderful support. Finally I thank all the committee members who have done their roles sincerely with dedication.

I am confident that the Course Manual released on this occasion would enable the participants to enhance their knowledge and competence in the area of mariculture and once they are back to their country they can contribute a part of it at least to their nation.

November, 2013

Imelda Joseph
Coordinator

FOREWORD

Capacity building is a cross cutting theme and is one of the essentials for sustainable development of any sector. The Central Marine Fisheries Research Institute (CMFRI) undertakes capacity building activities in marine capture fisheries and mariculture. These include provision of training courses by the Human Resource Development (HRD) Cell of the institute by conducting national trainings/ workshops with technical support to a cross section of groups including students, researchers, entrepreneurs including self help groups and fishermen, preparation of training materials (Technical Manuals, Brochures, Extension leaflets, Posters etc.) and conduct of national and international trainings/ workshops with funding from national and international organisations/ funding agencies on custom training courses on specific topics. The capacity building for in-house staff members including Scientists and Technical staff of the institute are also promoted within the institute as well as outside Institutions of International repute.

CMFRI has developed world class facilities in Mariculture Research at its headquarters at Cochin as well as at Regional Centres at Mandapam and Visakhapatnam and Research centres at Karwar and Vizhinjam. The institute has been successful in many mariculture technologies like breeding and culture of bivalves (pearl oyster, edible oyster and mussel), marine ornamentals, sea cucumbers and marine finfishes like cobia and pompano. The institute has also pioneered in open sea cage culture of a variety of finfish and shellfish species using indigenous cages and mooring systems.

CMFRI is emerging towards strengthening the capacity of men and women particularly small-scale entrepreneurs and fishers in the promotion and use of sustainable, cost effective and safe mariculture technologies developed by the institute, their socio-economic development, training and extension and information dissemination. These are being done through promotion of participatory approaches through technology demonstration and testing together with stakeholders.

I am happy to initiate a link with the Commonwealth Secretariat, London in capacity building for the Maldivian Officials in Mariculture. Bilateral relations between India and the Republic of Maldives have been friendly and close in strategic, economic and military cooperation. With this the Institute will become a centre for International trainings also. On the occasion, I acknowledge the efforts by my predecessor Dr. G. Syda Rao (Former Director) in showcasing the institute facilities to the international arena in a befitting manner. Mr. Mohammad Jasimudin, Acting Head of Regional Programmes Group at the Commonwealth Secretariat, London (U.K.) is gratefully acknowledged for the opportunity given to CMFRI for conducting the training. I thank Mr. Ismail Nishad, Human Resource Officer, Ministry of Fisheries and Agriculture, Maldives for liaising with the participants for the training. I am grateful to The Commonwealth Secretariat, London, U.K. for the funding. Support of the training is also derived from the partial NZAID funds received from the New Zealand Ministry of Foreign Affairs in support of the post-Tsunami capacity development of the fisheries sector of the Maldives. I thank all my colleagues at CMFRI and other organisations in contributing towards this training. Last but not the least I acknowledge Indian Council of Agricultural Research (ICAR) and Department of Agricultural Research and Education (DARE), New Delhi, for facilitating the training with timely clearances from the Ministries and necessary support. I am confident that the participants would greatly benefit from this month long training at CMFRI.

November, 2013

A. Gopalakrishnan
Director

Mariculture: An Overview

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Capture fisheries and aquaculture supplied the world with about 148 million tonnes of fish in 2010 (with a total value of US\$217.5 billion). With sustained growth in fish production and improved distribution channels, world fish food supply has grown dramatically in the last five decades, with an average growth rate of 3.2% per year in the period 1961–2009, outpacing the increase of 1.7% per year in the world's population. World per capita food fish supply increased from an average of 9.9 kg (live weight equivalent) in the 1960s to 18.4 kg in 2009, and preliminary estimates for 2010 point to a further increase in fish consumption to 18.6 kg. China has been responsible for most of the increase in world per capita fish consumption, owing to the substantial increase in its fish production, particularly from aquaculture (FAO, 2012).

It is well recognised that many of our exploited marine fishery resources have already reached the maximum sustainable levels and hence, increasing the fishing pressure to augment the marine fishery resources may not be a viable proposition. In this context, for meeting our future additional demand for seafood, it is inevitable to venture into mariculture practises. The development and standardisation of commercially viable mariculture activities is the major prerequisite. Mariculture involves the cultivation of marine organisms in seawater for food and other products either in the open ocean, an enclosed section of the ocean, or in tanks, ponds or raceways. Examples for mariculture include, the farming of marine finfish, shellfish e.g. prawns, lobsters or oysters, mussels and seaweeds. Non-edible products produced by mariculture include: fishmeal, nutrient agar, jewellery (e.g. cultured pearls), and cosmetics.

About 600 aquatic species are cultured all over the world in a variety of farming systems and facilities of varying input intensities and technological sophistication, using freshwater, brackishwater and marine water. Aquaculture activities other than for human consumption include live bait farming for fishing; live ornamental ani-

mal and plant species and ornamental products (pearls and shells); fishes cultured as feed for certain carnivorous farmed species; culture of live feed organisms such as plankton, *Artemia* and marine worms for use as feed in hatcheries and grow-out systems; aquaculture hatchery and nursery outputs for on-growing in captivity or stocking to the wild; and capture based aquaculture. Asia accounted for 89% of world aquaculture production by volume in 2010, up from 87.7% in 2000.

In the world scenario, contribution of India in mariculture production is very negligible. In other countries in the Asia Pacific region significant advances have been taken place in the development and expansion of mariculture. Mariculture sector is looked forward as the sector for increasing seafood production in the coming years in all the countries. The Central Marine Fisheries Research Institute (CMFRI) is the pioneer in mariculture research in India, and many technologies have been developed by the Institute during the last five decades. Initial focus was only in enhancing shellfish production. During 1970s the technology for mussel farming was initiated and standardized in the country. Commercial mussel farming gained rapid strides since 1996 in India. In the recent years mussel farming showed spectacular improvements with the farmed mussel production of the country reaching a total of about 20,000 tonnes. Though efforts to popularize the technology were undertaken in the States of Kerala, Karnataka, Goa, Maharashtra and Tamil Nadu a quantum leap in the mussel production was observed only in the state of Kerala mainly due to the preference of mussel meat in Kerala. The availability of large extent of natural mussels beds along the Indian coast for sourcing the seeds; high price realized for the produce in domestic market; minimal operational expenditure and short term eco-friendly farming techniques are expected to encourage more farmers to come forward to adopt the practice in future years. Edible oyster farming practised on a very small scale at certain locations in Kerala also requires to be

expanded. The two major concerns which have to be addressed are the low value - high volume production of spat to cater to the seed requirement and the development of suitable marketing channel.

During the 1980s technologies pearl production and artificial seed production of Indian white prawn *Fenneropenaeus indicus* were developed. Recently only it was felt that fish seed production as also essential for the country. The concerted efforts of more than a decade or so, CMFRI could achieve the seed production of cobia *Rachycentron canadum* and silver pompano *Trachinotus blochii* during 2009-10. Among crustaceans, shrimp has been produced in coastal ponds in the country and about 100,000 tonnes of American white shrimp *Penaeus vannamei* is produced in the country outpacing the tiger shrimp *P. monodon*. However, the two promising marine crustacean species are the blue swimmer crab *Portunus pelagicus* and the sand lobster *Thenus orientalis*. Though seed production of these species has been developed by CMFRI, commercial level seed production technology for both the species are yet to be achieved.

The marine ornamental fish industry has been expanding globally in recent years and about 20 to 25 million marine ornamental fishes are traded annually. Nearly 98% of the marine ornamental species marketed are wild, collected mainly from coral reefs of tropical developing countries. This has been demonstrated as a viable enterprise in India threatening the long term sustainability of the trade due mainly to indiscriminate exploitation of coral reef areas, leading to degradation of coral reef habitat and overexploitation of desired species. In this context The Central Marine Fisher-

ies Research Institute has been focusing on this aspect for the past few years and a variety of marine ornamental fishes have also been bred by the institute. Techniques for broodstock development, breeding and seed production of 12 species of pomacentrids were developed and standardized by CMFRI.

CMFRI has also pioneered in open sea cage culture during the last decade and has standardized cage design and mooring for Indian waters. Many species of finfishes like Asian seabass, cobia, mullets and pearlspot were successfully reared in cages in different maritime states of the country. Among shellfishes capture based aquaculture of spiny lobsters were found to be highly profitable. CMFRI has also set up a model for community development through cage culture as in the case for Sidi tribe from Africa, settled in Gujarat. It was developed as a social movement and the progress made in the community can be taken as a model for community development through PPP mode.

Since Maldives is a close associate of India, many of the mariculture technologies developed in India can be transferred easily. Capacity building is one such area where we can have association in the future also. Before going in a bigger scale, it should be borne in mind that mariculture activities should focus on development of sustainable and economically viable farming technologies, which can be easily adopted by the end user. Sustainable mariculture promises economic and environmental benefits. An object oriented development approach, coupled with appropriate policy formulations can lead to the emergence of mariculture as a substantial contributor to the seafood production of the country.

Brackishwater aquaculture in India – An overview

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Introduction:

The world fish production is around 152 million tonnes supporting the nutritional security of the growing population of the world. Out of the total fish production, aquaculture contributes around 42%. The capture fisheries, though intensive efforts are made for exploitation in many cases is static or declining. In some areas through continuous unregulated over exploitation it has often exceeded the MSY (Maximum Sustainable Yield) and the aquaculture has to necessarily support the fish production. Aquaculture is considered as one of the potential growth sectors showing annual growth rate between 8 and 10% and is dominated by Asian countries. India is in the second position after China; however, the contribution is only to the extent of 5% compared to that of 70% by China. The present total fish production in India is to the extent of about 7.8 million tonne of which, around 3.8 million tonnes is contributed by marine fish production including through coastal aquaculture and the rest is by the fresh water sector. The present production has to make a quantum jump in the coming years to meet the demand. The present per capita consumption of fish in India is around 9kg where the global average is in the order of 15kg. Considering the population of India, which will be around 1200 millions by 2020 and of which 60 % of the population will be fish consumers, the domestic need itself will be in the order of 11-12 million tonnes. Aquaculture is considered as a source for nutrition security, livelihood for million, provide employment, social security for improving the economic status and social upliftment in India. It would help in reducing the pressure on wild stock and culture of organisms lower in the food web like seaweeds and molluscs would help in environmental quality improvement. Aquaculture can also be integrated with other farming systems like agriculture, animal husbandry and dairying

Potential resources

India, with its long coastline of about 8,129 km intercepted with innumerable estuaries along

the coastline with vast stretch of brackishwater lakes like Chilka in Odisha, Pulicat in Tamil Nadu and Andhra Pradesh and Vembanad in Kerala, lagoons and creeks and backwaters has got great potential brackishwater resource for developing aquaculture. The resource include about 3.5 million ha of estuaries, 3.9 million ha of backwaters and 0.4 million ha of mangrove swamps. It has been estimated that around 1.19 million ha of area in the coastal brackishwater eco system is suitable for aquaculture. Apart from this vast stretch of inland areas to the extent of about 8.2 million ha is salt affected which are marginally suitable or unsuitable for agriculture having high potentials of ground saline water, notably in the states of Haryana, Rajasthan, Western Uttar Pradesh, Gujarat, Bihar and selected parts in other states. For the development of brackishwater a great biodiversity of fish and shell fish species showing high growth potential, greater adaptability, good market demand in the domestic and international markets with excellent flavour and taste are available for farming in the brackishwater ecosystem in India. The major groups amongst fishes include the herbivorous species like Grey Mullet (*Mugil cephalus*) and many other species of *Liza* (like *Liza tade*, *Liza partia*, *L. tracheli*, *L. macrolepis* etc., Milk Fish (*Chanos chanos*), Pearlsplit (*Etioplus suratensis*), Rabbit Fish (*Siganus* sp.) and high valued species which are mainly carnivorous like Seabass (*Lates calcarifer*), Groupers (*Epinephelus tauvina*, *E. coioides*, *E. malabaricus*, *E. fuscoguttatus* and Snappers *Lutjanus* sp., Carangids like Pompano (*Trachinotus blochii*), Cobia (*Rachycentron canadum*) are some of the candidate species identified for farming in India.

High valued shrimps like Tiger Shrimp (*Penaeus monodon*), Indian White Shrimp (*F. indicus*), Banana Shrimp (*F. merguensis*) and Exotic White Legged Shrimp (*Litopenaeus vannamei*) are some of the shrimp species farmed in India adopting different practices under varied conditions. Mud Crabs (*Scylla serrata*, *S. tranquebarica* and *S. oceanica*) are farmed by small aqua farmers for sustenance in brackishwater environment.

Status of brackishwater aquaculture:

Brackishwater aquaculture is traditionally practiced in India in the low lying fields of Kerala (Pokkali), West Bengal (bheries and gheries), Odisha, Goa (khzan) and Karnataka (kar) which experiences influx of salt water. The practice is just allowing juveniles of fish/shrimp those are found in the brackishwater estuaries, creeks or backwaters brought by the tidal waters into the fields and allowing them to grow feeding on the organisms that enter into the culture system. They are not provided with any supplementary feed. The water exchange is facilitated through tidal waters. Harvesting is periodically done and the practice continuous for 4-5 months in seasonal fields and throughout the year in perennial fields. The production and the productivity (is in the range of 300-400kg/ha).

With the improvement of technologies and the necessity of aquaculture, these practices were improved with supplementary stocking and/or feeding, water quality management, health management and maintenance aiming at higher production. The Indian brackishwater aquaculture slowly switched over from the traditional farming system to improved farming system (semi intensive). After the demonstration of fish/shrimp farming through All India Coordinated Project on brackishwater aquaculture in 1970s, the aquaculture sector found new opening with the advent of seed production technologies and establishment of feed mills opening new vistas for the scientific farming. Following all the protocols for farming, production ranging from 2 to 20 tonnes/ha mainly shrimp is obtained in a culture period of 4-5 months in the coastal area ponds. The technology advancement helped in the establishment of more than 380 hatcheries with a production capacity of 5-300 million seeds totalling around 20 billion and more and new areas were brought under shrimp farming. The present area of operation in the coastline is around 160,000 ha and producing around 200,000 tonnes of shrimp.

The brackishwater aquaculture which witnessed a phenomenal growth during 1980s and in the mid of 1990s has to face a set back later part of 1990s due to many socio-economic and environmental issues coupled with the outbreak of uncontrollable diseases like White Spot Syndrome Viral (WSSV) disease on shrimp. The

reasons attributed for this are the unregulated development and dependence on a single group of organisms (shrimp) for farming. The effect of this has brought the pronounced impact on the farming sector questioning the very sustainability of the coastal aquaculture.

Many options are put forth for sustaining the brackishwater aquaculture industry. Since, the viral diseases is transmitted both vertically and horizontally to reduce the transmission, SPF broodstock development/import was suggested and in this direction limited success has been achieved. For restricting the transmission of disease through environment improved farming practices (BMP, GMP) where advocated. These measures are expected to help in improving the coastal aquaculture. But, the recent problems like Early Mortality Syndrome (EMS) and the Slow Growth Syndrome (SGS) are making these issues more complex. One of the easiest options for the sustainability of the aquaculture can be diversification of species and practices of farming.

Culture of crustaceans:

Shrimp culture

Farming of high valued species of crustaceans is the main activity in the brackishwater aquaculture. Traditional farming of shrimp like Tiger Shrimp (*Penaeus monodon*), Indian White Shrimp (*F. indicus*), Banana Shrimp (*F. merguensis*), etc. were carried out in the tidal farms. These farms are in bounded ponds in the low lying brackishwater areas of Kerala, West Bengal, Goa, Karnataka and Odisha. This practice is done depending upon the natural water sources, feed and seed which is still in vogue in these areas. In this practice impoundments are provided with water inlets (sluice gates) to regulate the water. The brackishwater that enters into this impoundment are the source of seed and the species available will dominate in the farming. Seeds may include many species of fish and shell fish which may grow fast or slow. In this type of practice productivity was very low, not more than 400kg/ha.

With high export demand for the high valued crustaceans, brackishwater aquaculture emerged as an important farming system (semi intensive) where desirable species seed is stocked in known quantity and other inputs like feed are manipulated. Efforts are also made to provide desirable

quality water. The aquaculture which was traditional, emerged as a high input and high profit oriented activity. New areas where no farming was practiced earlier were brought for brackishwater farming. This practice of improved farming emerged as a major activity from the mid of 1980s and the farming was synonymised with the single species, the Tiger Shrimp. In some places like Kandaleru creek in Andhra Pradesh these activities were highly concentrated. The activity which was showing phenomenal growth for a decade (upto 1994) faced a set back due to the outbreak of uncontrollable viral diseases like White Spot Syndrome Virus (WSSV) coupled with many other social and environmental issues. Brackishwater farming required regulations for the better management of the farming system as well as the surrounding environment.

To control the outbreak of diseases all out efforts are made in understanding the etiology, diagnosing the disease and overcome the problem. Since the disease spread both vertically and horizontally, preventive measures for controlling vertical transmission are taken by developing SPF (Specific Pathogen Free) broodstock of shrimp and to control the horizontal transmission Better Management Practice (BMP) with bio secured environmental conditions are suggested for adoption. Since full proof SPF stock could not be developed in India for indigenous shrimp species like Tiger Shrimp (*P. monodon*) and motivating farmers for adoption of BMP is difficult since 80% of the farmers have small holdings other options become important to be considered. Due to the problems in sustaining the shrimp farming an interim arrangement has been made with the import and introduction of exotic species *L. vannamei*, SPF stock is being attempted. The stock is quarantined and regulated seed production activity and farming practices are suggested and this has paid dividend in safeguarding the shrimp culture with increased production. The quantum jump unit production of *vannamei* with high input may need many difficult options for sustaining. The regulations and their adoptions like BMP may be required for the ecological safety and security of the crop in India.

Mud crab culture:

Mud crab belonging to the genus *Scylla*, (*Scylla tranquebarica*, *S. Serrata*, *S. oceanica*) has

emerged as an activity for the small scale aqua farming in the brackishwater eco-system since mud crab can be farmed in small areas with relatively easy monitoring of water quality, etc., crab farming has emerged as an activity of livelihood for small scale farmers and Self Help Groups. Crab culture is done in small tanks, ponds, pens or in larger shallow water bodies as well in cages. Crab culture is being done as a) crab fattening, b) crab culture from juvenile to marketable size.

In fattening practice, moulted (water crabs) ones which are not suitable for marketing and won't fetch high price are procured from landings. These are carefully transported and released in ponds / tanks / pens depending upon availability and the capacity of the farmer. These crabs are fed with chopped low cost fishes for a period of 20-30 days and after they become hard shelled, marketed for premium price. For example, a water crab may fetch around Rs.100/kg and after hardening, depending upon the size of the crab, it will fetch price ranging from Rs.500 to Rs.1500/kg. However, the availability of water crabs is a major limiting factor for expansion of the crab fattening.

Juvenile crabs collected from the brackishwater environment (size of 5-10g) or crablets procured from the hatchery are reared in nurseries for a period of 2-3 months till they reach a size of 20-30g. Then, they are stocked in the grow-out system and reared for 4-5 months till they reach marketable size of more than 500g. This practice is yet to gain momentum because of longer culture duration and less survival rate. The seed availability is also limited and the commercial hatcheries are yet to be started in India for providing crablets. The other method of crab culture is mainly keeping in mind the selected clientele for quick chilled crab. In this practice, crabs are reared individually in cage and fed with trash fish or formulated feed and as soon as the crabs moult, they are picked up. These freshly moulted ones are chilled for further processing and marketed. This is a highly skilled operation and requires sophisticated infrastructure facilities.

Diversification to fish culture

Indian brackishwater aquaculture, though in the initial phase, have not aimed at any specific species or group of organisms, later has emerged with the orientation for particular group (shrimp).

However, the experiences gained for the past three decades have shown that for a sustainable aquaculture supporting better production diversification to other groups of organisms like fishes is highly desirable.

Most of the species which can be commercially farmed in brackishwater eco system are suitable for farming in marine and fresh water also. Some of the candidate species identified suitable for commercial aquaculture are Seabass (*Lates calcarifer*), Groupers (*Epinephelus* sp.), Cobia (*Rachycentron canadum*), Pearspot (*Etroplus suratensis*), Milk Fish (*Chanos chanos*) and Grey Mullet (*Mugil cephalus*). For the aquaculture development and expansion, the most important pre-requisites are the seed and feed. Seed production technologies have been developed for some species like Seabass, Cobia, Pampano and Pearlscale and for other species like Groupers, Snappers, Grey Mullet and Milk Fish, efforts are made by different R&D Institutions in India to develop and standardize seed production technology. For brackishwater fish farming feed has been developed for species like Seabass which has been tried and proved to be viable under pond culture system. However, the commercial cost effective feed is yet to be developed for brackishwater fish culture.

Status of seed production technology and culture of some brackishwater fishes

Asian seabass

Seed production technology

Comprehensive technology for controlled breeding of seabass was developed in 1997 and since then the technology has been further refined and validated. The technology includes captive broodstock development, acceleration of maturation, providing optimum conditions like water quality management, health management and feed management, induction of spawning through hormonal administration and facilitating natural spawning in the Recirculatory Aquaculture System (RAS). Larvae are reared feeding with live feed like Rotifers up to 9th day followed by *Artemia* nauplii up to 20 days and afterwards weaned to formulated diet or shrimp/fish meat. The fry are further reared in nurseries upto 45 days or so and are used for farming in cages or ponds.

Farming

Traditional farming

Seabass is cultured in the ponds traditionally as an extensive type culture throughout the areas in the Indo-pacific region where seabass is distributed. In low lying excavated ponds, whenever the seabass juveniles are available in the wild seed collection centers (For eg. April-June in West Bengal, May-August in Andhra Pradesh, Sept-Nov. in Tamil Nadu), May to 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. Forage fishes like Tilapia will also be available in these type of ponds. 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 are introduced into the pond at times of food scarce, 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 fishes. This is a common scenario in many coastal areas. In this manner production up to 2 ton/ha/7-8 months have been obtained depending upon the number and size of the fishes 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 the 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.

Culture of seabass feeding with low cost fishes

Seabass seed can be stocked in a prepared pond @10000/ha. The seed size of 2.0 gm and above is preferable for stocking in the growout farms. Water depth should be maintained not less

than 1.0 M. Seabass fishes stocked can be fed with minced meat of trash fish. Cheaper fishes like Tilapia, Sardines, horse mackerels which may not fetch more than Rs.5/- per kg can be bought from the commercial fish landing centers, washed and freezed in cold storages as required. The fish can be taken out an hour/prior to feeding, thawed and minced as meat using meat mincer. Feed can be made as dough ball like paste and placed in trays, kept hanging in 4 or 5 places in the pond. Feeding rate is ad libitum in any case not more than 100% body weight on wet weight basis of the biomass initially and gradually reduced to 10% at last phase of culture period. Feed rations can be given in two doses in the fore noon and after noon.

Fish farming with formulated feed

Seabass is cultured with extruded floating pellets in Australia, Thailand, Malaysia and Singapore. Being a carnivorous fish seabass needs high protein diet. Normally, in the preparation of diet for seabass, the animal ingredients are added more than 60% so that the required protein levels can be kept. The nutritional requirements of the Seabass are as follows: Protein around 55%, Lipid-15%, Fatty Acids-2%, Carbohydrates-15%. Since, Seabass is a fish feeding mainly on the fishes and shrimps moving in the water column (pelagic); the pellet should be slow sinking and should be in the column for reasonable time so that the fish can ingest the food before it reaches the bottom. The extrude pellets will have reduced loss; the digestibility will be good due to pre cooking, the feed mixture can be with higher moisture, the flavor of feed also can be retained with addition of excess fish oil. The pellet size should be from 2.0 to 6.0 mm as per the size of the fish.

The major constraint in the adoption of seabass culture in the brackishwater system is the longer duration of culture (9-10 months) since the brackishwater aqua farmers are tuned to have the harvest of shrimp within 5 months culture duration. In order to motivate the farmers efforts were made to reduce the culture duration by phasing out the culture period in three phases as a) Nursery phase, which will take about a period of two months to get a fish of 5-6g, b) Pre-grow out phase where the fingerlings grown to juveniles (50-60g) involving a duration of two months and c) the Grow out pond culture for a period of 5-6 months

to get a marketable size of 700-1000g fish. The advantage of this for a nursery and pre-grow out phase was the requirement of space is less and this can be concurrently carried out in the culture system itself.

Culture of seabass in cages

Fish culture in cages has been identified as one of the eco-friendly at the same time intensive culture practice for increasing in fish production. Cages can be installed in open sea or in coastal area. The former 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 as 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, assures high survival rate. It is natural and eco-friendly and can be adapted to any scale. Feeding can be controlled and cages can be easily managed. Fishes in the cages can be harvested as per the requirement of the consumers, which will fetch high unit price. Cage culture though involves little more capital inputs, the operating cost are minimum.

In the cages, fishes can be stocked @25-30nos/m² initially when they are in the size of 10-15g. As they grow, after 2-3 months culture, when the fish attained a mean body weight of 150g stocking density has to be reduced to 10-12 nos/m² for space. Cage culture is normally done in two phase – till they attain 100-150g size in 2-3 months and afterwards till they attain 600-800 in 5 months.

Fishes 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. Since, a lot of low cost fishes are landed in the commercial landings in the coastal areas which are fetching around Rs.3-5/kg only used as feed for seabass culture. Low cost fishes like also serve as feed for seabass in ponds and in many cage culture operations. The rate of feeding can be maintained around 20% initially and reduced 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 feed conversion ratio (FCR) works out around 6 or 7. In the case pellet feeding FCR is to be 1 to 1.2 in Australia. However, the cost effectiveness of the pellet feeding for seabass in grow out culture has to be tested.

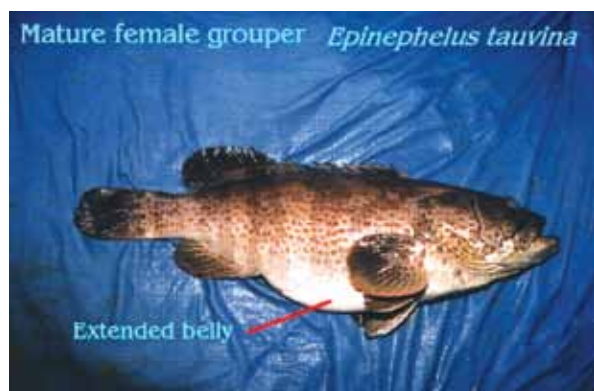
Groupers

Groupers also migrate for maturation and spawning to deeper waters in the sea. The groupers attain maturity after 2 years at their age when they are around 2-3 kg in size. Groupers are protogynous, herbivorous where many are females in the early period and reverse to male when they are larger in size. In hatchery operations, for obtaining male some times require intervention through exogenous hormone administration. Successful breeding of some species of groupers have been reported from different R&D Institutions like CMFRI, CIBA and RGCA. The techniques for reverting female to male and retaining them as male has been developed in CIBA through oral administration (through feed) of 17 methyl testosterone hormone in the dose @ 2mg/kg body weight at on every alternate days. The breeding protocols in-

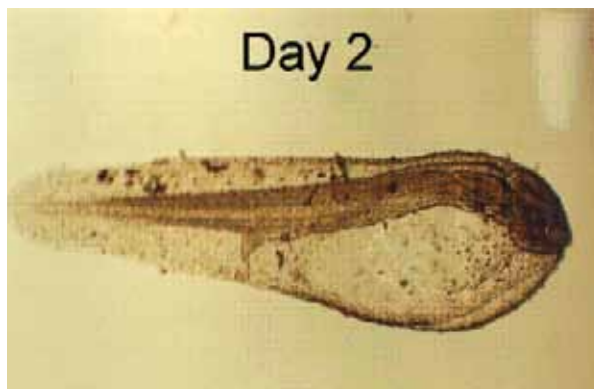
clude the selection of females with ova diameter of above 450 μm and administration of HCH hormone @ 750-1000IU /kg body weight for females and LHRHa @ 40 $\mu\text{g/kg}$ body weight. Successful spawnings were observed after 72-144 hours of hormonal administration. Hatching took place after 22-24 hours of incubation. Rearing the larvae feeding with rotifers SS strain where the size is less than 80 μmm following green water technology has been succeeded. However, survival rate is very less (around 5%) in many cases for a months rearing. Though Grouper culture in an organized manner has not been taken up, trials are being carried out by various R&D institutions on the viability of culture in cages and ponds.

Grey mullet (Mugil cephalus)

Grey Mullet *Mugil cephalus* is a herbivorous fish. Considering its high potentiality for farming along with other fishes and shell fishes with low cost inputs the good market demand in some parts of India like Kerala, West Bengal. It is felt that it will be highly useful for a sustainable farming in traditional coastal farms. However, breeding of grey mullet under controlled conditions, though being attempted for some years, is yet to take off as a standardized technology for commercial venture.



Gravid Fish ready for spawning



Hatched out larvae

Grey mullet in the size of 300 g- 1.5 kg collected from the wild catch or farm reared stock could be maintained in earthen ponds or broodstock holding tanks feeding with formulated feed @ 2-3% of body weight daily providing with quality sea water with the desirable parameters prevailing in the open sea and taking care of the regular health monitoring protocols. Matured fishes could be obtained during the spawning season, normally in the months of October-January. Breeding protocols include selection of females with ova diameter around 0.58 mm-0.6 mm and administration of a prime dose of HCG @ 1000 IU and a resolving dose of LHRH @ 40-50 µg/kg body weight and half the dose for the males was found to make successful spawning. The larvae also could be reared following the protocols as for other marine fish larvae. In India though success in captive broodstock development, maturation and spawning has been achieved, the technology for commercial venture is yet to be developed. Grey Mullet culture is practiced in a more extensive way as a poly culture along with other fish and shrimp species. Experiences have indicated

poly culture of shrimp and Mullet is desirable to reduce the risk of shrimp disease outbreak since Grey Mullet as a detritus feeder is useful in improving the eco-system condition on reducing the shrimp pathogens.

Milk fish (Chanos Chanos)

Milk fish breeding and seed production has become a house hold activity in countries like Philippines, Indonesia and Taiwan. However in Indian context, breeding of milk fish under captivity is yet to make a beginning. Captive broodstock of milk fish developed after feeding them with formulated feed @ 2-3% body weight after 5 years of holding under captive conditions have shown male maturation and the female fishes have not attained gonadal maturity.

Milk fish culture in India is being carried out along with shrimp as poly culture and mono culture of Milk Fish is tried. The market price for milk fish is less compared to other fishes the cost effective farming system has to be developed with low cost feed and farm management.



Milk fish brooders



Pearlscale (Etroplus suratensis)

Pearlscale (Etroplus suratensis)

Pearlscale (Etroplus suratensis) an indigenous chichlid having a high market value in some parts of India like Kerala is considered as a highly suitable table fish which can be farmed in ponds or cages with low input in shallow/freshwater/brackish water systems. Pearlscale breeds in the confinement. After pair formation selecting a suitable hard substrate for the egg are laid in a mosaic manner by the female and fertilized by the sperm released by the males by following the course of the female. Eggs are guarded and cleaned periodically for a period of 6-7 days after which they are transferred to nests (pits), at the time of hatching; the hatchlings subsist with yolks for 3-4 days after which the hatchlings are guarded by the parent fishes till they attain the advanced fry or fingerling stage. To increase the survival rate in the early stages, the eggs at the time of hatching is transferred to tanks and maintained with good aeration through which the hatching rate is improved. Afterwards the juveniles are fed with live zooplankton initially and/ or later with egg custards and formulated feed.

Due to the high value Pearlscale could fetch in some parts of India farming of this table fish which is considered as a delicacy is practiced traditionally especially in Kerala. This indigenous chichlid fish can be bred in confined waters. However, producing large quantity of seed in a single place poses problem due to the low fecundity and involves large number of broodstock management. Breeding and seed production in small units in large numbers may be useful in solving the problem. The state of Kerala has given priority to Pearlscale farming and conservation and lot of efforts are made under various schemes for promoting home state pond culture system which will serve as a livelihood option for thousands of fisher folk in increasing fish production.

Background, Global trend and Types of Mariculture

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Introduction

Fisheries and aquaculture are important sources for food and livelihoods for more than one billion people in the world. The waterways and oceans cover about two thirds of the surface of the earth and it forms the most underutilised natural resource when it comes to food production. The fact that aquaculture is the world's fastest growing food production technology indicates not only that one has started to exploit this potential but also if man succeeds in using the oceans more efficiently, aquaculture can be the largest single contributor to less pressure on land.

Background

Aquaculture is distinguished from other aquatic production such as fishing by the degree of human intervention and control that is possible (Anderson 2002). The production process in aquaculture is determined by biological, technological, economic, and environmental factors. However, the key factor is that many aspects of the production process can be brought under human control. This control makes innovation possible and is, accordingly, essential for the rapid technological development that has fuelled production growth since the early 1970s. As defined by the United Nations Food and Agriculture Organization (FAO), aquaculture is the "farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants. Farming implies intervention in the rearing process to enhance production, like stocking, feeding, security measures etc. The advent of aquaculture dates back millennia, though its exact origins are unknown and a large proportion of organisms that humans rely on for protein and sustenance come from the sea. Currently, approximately 16% of the animal protein consumed by the world's population is derived from fish, and over one billion people worldwide depend on fish as their main source of animal protein.

In the 1970s, what is sometimes labelled as the "blue revolution" began as humanity's accumulated knowledge of aquaculture allowed for

the introduction of semi-intensive and intensive farming practices. As a result, producers were able to influence the growing conditions of the fish through feeding, breeding, and so forth, and the production cycle was closed for an increasing number of species. The increasing control of the production process enabled a number of productivity-enhancing innovations to take place. Improved productivity resulted in a reduction in production costs, and with a given price, this led to more profitable production. A number of species are being farmed in all parts of the world, in freshwater and in saltwater. Moreover, a number of different production techniques are being used, adapted to different species, environments, and economic conditions. These techniques include ponds, pens, raceways, ropes, cages, tanks, and closed circulation systems.

Trends in aquaculture

While the growth potential for wild fisheries is limited, it is vast for aquaculture. Aquaculture is a production technology with its origin in Egypt and China thousands of years ago. Beginning in the 1970s, a significant change took place as better control over the production process enabled to develop a number of new technologies and production practices. These changes dramatically improved the competitiveness of aquaculture products both as sources of basic food and as cash crops. The combined effect of productivity and market growth has made aquaculture the world's fastest growing animal-based food sector of the last decades (OECD, 2010).

The species produced in aquaculture is almost as large as in wild fisheries. Aquaculture production includes kelp (seaweed), mussels, crustaceans, carps, tilapia, salmon, seabass, shrimp etc. While aquaculture has been a success in terms of increased production, it also faces strong opposition in many countries because the new technologies that are enabling the increased aquaculture production are interacting negatively with the environment. There are numerous examples of unsustainable as well as sustainable aquacul-

ture practices. It is of the highest importance to encourage sustainable practices and discourage ruining of locations and causing negative impacts on the environment.

Production

Aquaculture is a truly global production technology, with about 180 countries reporting some level of production. However, there are substantial regional differences. Asia makes up about 92% of the production measured by volume and 79.6% by value. All the other regions have a higher value than volume share, because they produce high value products especially South America. China is by far the largest producer country, with a value share of more than 50% and a volume share of 70%. Measured by value, India, Chile, Vietnam, Japan, Norway, Indonesia, Thailand, Burma, and South Korea are the other top 10 producing countries. Egypt is the largest producer in Africa and is ranked number 13. Aquaculture is clearly strongest in Southeast Asia and is primarily conducted in developing countries.

The total supply of seafood increased from 69.0 million tonnes in 1976 to 142 million tonnes

in 2008 (FAO, 2011). Hence, the availability of seafood has more than doubled during this period. Seafood appears from two main modes of production – harvest and aquaculture, and the development of production in total capture and culture production since 1970 is shown in Fig.1. Until the 1970s, though aquaculture was not very important, a virtual revolution has taken place since then. In 1970 aquaculture production was still rather miniscule with a produced quantity of about 3.5 million tonnes, representing 5.1% of total seafood supply. In 2006, it was made up to 41.8% with a production of 66.7 million tonnes. The increased production in aquaculture is accordingly the only reason why global seafood supply has continued to increase since 1990. Stimulated by higher demand for fish, world fisheries and aquaculture production is projected to reach about 172 million tonnes in 2021, with most of the growth coming from aquaculture. Aquaculture will remain one of the fastest-growing animal food-producing sectors (SOFIA, 2012). The aquaculture production from 2006 is given below:

Aquaculture Production (Million tonnes; FAO, 2012)

	2006	2007	2008	2009	2010	2011
Inland	31.3	33.4	36.0	38.1	41.7	44.3
Marine	16.0	16.6	16.9	17.6	18.1	19.3

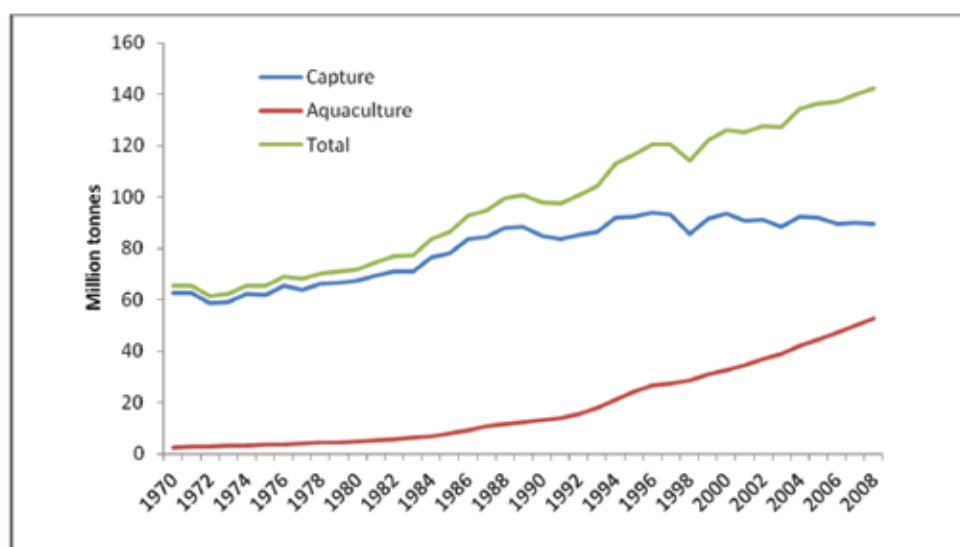


Fig.1. Development of fisheries production in total capture and culture production since 1970 (Source: FAO)

The world mariculture production is like; Molluscs (23.6 %, 14.2 million tonnes); Crustaceans (9.6 %, 5.7 million tonnes); Diadromous fishes (6.0 %, 3.6 million tonnes) and Marine fishes (3.1 %, 1.8 million tonnes) with a total of 29.2 million tonnes.

Salmons represent the largest diadromous fish species group, with an average growth rate of 5.5% per year over the last decade. Trouts represent the second-largest diadromous fish species group, with an average growth rate of 3.5% per year over the last decade. Milkfish represent the third-largest diadromous aquaculture species,

with species production growing at an average rate of 4.7% per year over the last decade. Eels represent the fourth-largest diadromous aquaculture species group, with species group production growing at an average rate of 2.8 % per year over the last decade. Marine fishes represent the last major fish species group by production, with species group production growing at an average rate of 8.1 % per year over the last decade. Marine shrimps represent the largest crustacean species group, with species group production growing at an average rate of 14.7% per year over the last decade (FAO, 2010).

The major cultured fish and crustacean are:

Marine crustaceans: 3.64 million tonnes, valued at US\$15.0 billion

- Shrimps – 3.40 million tonnes, six major spp.
- Crabs – 241 000 tonnes; one major species

Diadromous fishes: 3.26 million tonnes, valued at US\$12.95 billion

- Salmons – 1.57 million tonnes, two major spp.
- Trouts – 677 000 tonnes, one major sp.
- Milkfish *Chanos chanos* – 676 000 tonnes
- Eels – 265 000 tonnes, one major sp.
- Miscellaneous diadromous fish species – 71 000 tonnes; one major sp.

Marine fishes: 1.77 million tonnes, valued at US\$6.6 billion:

- Seabass – 214 000 tonnes, two major spp.
- Mulletts – 235 000 tonnes, one major spp.
- Porgies, seabreams – 253 000 tonnes, two major spp.
- Jacks, crevalles – 184 000 tonnes, one major sp.
- Flounders, halibuts, soles – 149 000 tonnes, two major spp.
- Croakers, drums – 123 000 tonnes, two major spp.
- Groupers – 78 000 tonnes;
- Cods, hakes, haddocks – 21 387 tonnes, one major species;
- Tunas, bonitos, billfishes – 8 926 tonnes, one major species; and
- Miscellaneous marine fish species – 499 000 tonnes, three major species

On a global basis, more than 85.5% of fish and crustacean aquaculture production was produced in the Asian continent in 2008 (26.9 million tonnes), followed by the Americas (1.93 million tonnes, or 6.1%), Europe (1.64 million tonnes, or 5.2%), Africa (0.94 million tonnes, or 3.0%), and Oceania (50 317 tonnes, or 0.2%; FAO, 2010a). Twenty countries accounted for 94 % of total global fed fish

Country	Production (million tonnes)	Percent of total production
China	15.67	49.8
India	3.08	9.8
Viet Nam	2.12	6.7
Indonesia	1.64	5.2
Thailand	1.03	3.3
Norway	0.84	2.7
Philippines	0.70	2.2
Egypt	0.69	2.2
Myanmar	0.65	2.1
Chile	0.63	2.0
Bangladesh	0.62	2.0
United States	0.34	1.1
Japan	0.30	1.0
Brazil	0.27	0.8
Taiwan		
Province of China	0.22	-
Ecuador	0.17	-
Malaysia	0.17	-
Turkey	0.15	-
Mexico	0.14	-
United Kingdom	0.14	-

and crustacean production in 2008, with China alone accounting for about half of the global total (Table). These top 20 fed species producers were also the largest consumers and producers of feed, either in the form of fresh feeds, farm-made feeds or commercial feeds.

What matters for the development of aquaculture is the degree of control of the production process. It is this control that enables innovation and systematic gathering of knowledge that creates further growth. As such, it is the transition from extensive to semi-intensive farming in South-east Asia, and in particular the feeding of the fish, that is the most important factor for the growth in aquaculture production. As species with highly intensive production systems lead the way, the production process is likely to become even more intensive in most places.

Types of mariculture

Mariculture of a new species typically starts by catching wild juveniles and feeding them in

a controlled environment. As more knowledge is gained, the degree of control with the production process increases and the farmers can increase their influence on growth and reproduction. The degree of control is often categorised by the intensity of the aquaculture operation. Traditional aquaculture varies between extensive and semi-intensive farming practices. Mussel farming is an example of an extensive method used around the globe, whereby the farmer provides a rope or a stake for the mussel fry to fasten to and undertakes some culling so that the density does not get too high, but otherwise leaves the mussels to grow without further interference. The small ponds used in Chinese aquaculture were traditionally operated on an extensive basis, because the farmer did little to control growth and biomass. In intensive aquaculture, the production system is closed so that one does not depend on wild fish for reproduction.

Aquaculture production systems and practices, by region (Source: FAO)

Region	Major Culture Species	Major Culture Systems	Major Culture Practices	Scope for Future Development/Needs for Further Expansion
ASIA	At least 75 species; diverse freshwater and marine species, including high-value shrimps, molluscs, seaweeds, with carps and seaweeds dominating production	Traditional extensive to intensive	Fish ponds Fish pens and fish cages Floating rafts, lines, and stakes for molluscs and seaweeds	Development of culture-based fisheries in inland lakes, rivers, floodplains, and permanent and temporary reservoirs and barrages
				Resource enhancement programmes integrated with environmental management
PACIFIC	Mussels and oysters, red seaweeds	Intensive/semi-intensive to extensive	Hanging lines for mussels and pearl oysters	Production of high-value species for select markets;
			Offshore cages for salmon	Small-scale aquaculture for local markets;
			Pond culture for shrimps, tilapia, catfish, milkfish	Improved management of fishery resources, particularly reef fisheries
			Freshwater pens for crayfish	
LATIN AMERICA	50 species of fish, crustaceans, and molluscs, including freshwater fish and marine shrimps in South America and molluscs in Central America	Extensive to semi-intensive and Intensive	Offshore cage farming of Pacific and Atlantic salmon Ocean ranching in Southern Ocean Semi-intensive farming of marine shrimp in coastal ponds	Production of species for export and marine shrimp and salmon
MEDITERRANEAN	most important being salmonids and oysters and mussels	Well-diversified modern practices, with highly technical and intensive systems in developing countries and semi-intensive and extensive elsewhere	- Fish cages - Ocean ranching	Production of high-value species of tourism and export Integrated coastal zone management
CARIBBEAN	marine shrimp and oysters and seaweeds		Floating cages in reservoirs	Priority is for aquaculture production for local markets
			Rope production of molluscs	

- Marine Ponds are mainly for growing prawns and some finfishes either by tide fed systems or by pumping in off seawater at periodic intervals.
- Tank farming (Prawn broodstock tanks; Prawn culture tanks; Barramundi): Some species grow well in tanks which are aerated and have a continuous exchange of water to keep the dissolved oxygen levels high and remove wastes
- Sea Cage farming (Salmon, Tuna; Snapper, Barramundi, grouper):
- Long Line farming (Pearl oysters, Mussels): It uses a series of floats arranged in a row. The long-line is secured at each end with two anchors. One long-line is 100 m long and consists of about 51 floats connected by a polyurethane rope 15 mm in diameter. A series of strings of oysters called “rens”, each about 5m long is attached to each rope
- Raceway farming (Abalone; Oysters; Algae; Barramundi): Raceways are usually large concrete tanks; generally 30 m long, 3 to 10 m wide and 1 m deep and usually have higher flow rates than ponds.
- Fish hatcheries: Fish hatcheries are used to breed a large number of fish in an enclosed protected environment. Such an environment greatly increases the chances of survival of the fish fry. Many hatcheries then sell the juvenile fish for release into the ocean (e.g. into sea cages).
- Polyculture and integrated aquaculture: Polyculture and integrated aquaculture are methods of raising diverse organisms within the same farming system, where each species utilizes a distinct niche and distinct resources within the farming complex (Figure 2).³⁹ This may involve the rearing of several aquatic organisms together or it could involve raising aquatic organisms in conjunction with terrestrial plants and/or animals. In either case, the wastes from one organism are used as inputs to another, resulting in the optimal use of resources and less pollution overall. Polyculture systems can provide mutual benefits to the organisms reared by creating symbiotic relationships while allowing for a balanced use of the available aquatic resources, where-

as intensive monoculture systems extract resources from the system and place more stress on the surrounding environment. In addition, integrated systems can increase the economic efficiency of fish farms through improved conversion rates of input materials.⁴¹ Polyculture and integrated aquaculture have the potential to address some of the problems that arise from the intensive rearing of single finfish species. For example, the integration of fish culture with the culture of algal and/or shellfish species shows potential for reducing the risks of eutrophication and also for exploitation of the large amounts of wastes produced by fish farms. Further research is needed however, to determine the effectiveness of such systems, especially in open marine environments.

Closed and low discharge systems

Recirculating systems: Concerns for water conservation and reduced waste discharges have prompted the increased use of closed recirculating aquaculture systems. Recirculating systems generally consist of land-based tanks with constantly flowing water. The systems are made up of three basic components: culture chamber, settling chamber, and biological filter. Water enters the culture chamber, flows through the settling chamber and then moves through the biological filter to remove additional particulate matter. The water is then circulated back through the systems' culture chambers. Recirculating systems conserve water and allow producers to control all of the environmental factors that might affect their plants and animals. For example, aquaculturists have complete control over temperature, salinity, oxygen, predators and introduction and transfer of diseases. Recirculating systems, however, can be costly to operate, as they are highly dependent on electricity or other power sources. Pumps must be used in order to maintain the constant flow of water and often water must be heated or cooled to the desired temperature. Recirculating systems have less of an impact upon the environment because of their closed nature – wastes and uneaten feed are not simply released into the ambient environment in the manner that they are with net pens and exotic species and diseases are not introduced into the environment. In recirculating systems, wastes are filtered out of the culture system and disposed of in a responsible manner. Recirculating systems can be built just about

anywhere, including in urban settings where they can use existing structures and be placed close to markets, thereby reducing transportation costs. Recirculating systems can be used to grow a wide variety of fish species year-round in controlled environments.

Conclusion:

Maldives with a coastline of 644 km, by initiating mariculture with minimum inputs in the country can contribute to the economy as well and establish it in the near future with production of quality products within and outside the country.

Significance of Ecosystem Approach to Aquaculture

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Traditional eco-friendly farming practices

Aquaculture was practiced in several coastal areas of the world by simple methods such as collecting seed from naturally abundant areas growing them to harvestable size in coastal ponds. Simple supplementary feed using locally available natural resources were used and the production rates were moderate. The aqua farmers were satisfied since investments were low, mass mortalities of stocked resources were rare and there was moderate profit. These traditional systems in Asia especially in China and Vietnam have been productive for more than 3000 years. These eco-friendly aquaculture practices like paddy cum fish culture have benefitted several millions of rural people in Asia and have been designated as a “Globally Important Agricultural Heritage System”.

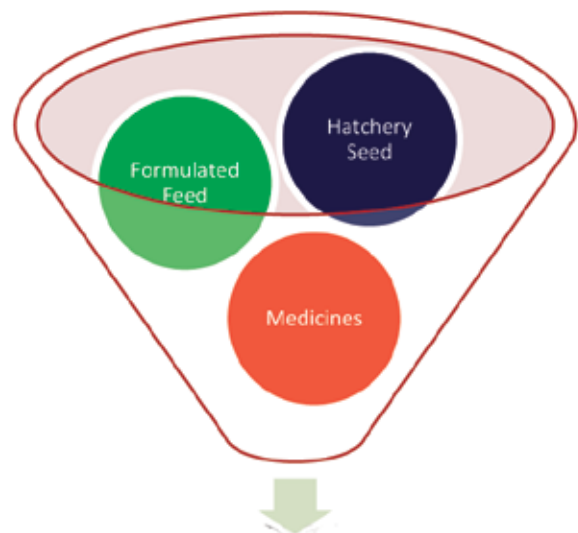


Development of modern aquaculture

With the increase in human population, the need for farmed fish increased and accordingly farming systems were modified and new systems were developed. Research on inputs required for increasing the productivity of aquaculture such as feed and seed increased and great strides were

made in seed production through controlled condition in hatcheries and feed production technologies using varied raw material. Thus the traditional simple aquaculture system began to be replaced by controlled farming methods such as the semi-intensive / intensive type of farming systems where resources are stocked in high densities and farmed under controlled conditions.

Globally, Asia continues to be the leading aquaculture production region with more than 85% of production. Aquaculture provides livelihood to nearly 17–20 million aquaculture farmers in Asia and it is important that the farming systems are sustained. That is, they should continue to flourish and be productive and provide the food and financial security to the farmers. However, unplanned growth and farming without considering the ecological potential of the farming area has lead to several negative impacts both to the farm and also to the natural ecosystem.



High production & negative impacts on environment if good management practices are not followed

Ecological Signals of alarm

There are clear examples of ecological damages when farms are constructed in the same location without taking into consideration the ecological carrying capacity or the potential. One example is that of Sandu Bay, a semi-enclosed bay with an area of 263 sqkm where yellow croaker, farming was started in 1995. Qingshan region was the main cage farming area in this bay and there were about 1000 fish cages. However, the successful farming operations prompted the farmers to increase the farms each year and by 2005 the number of farms increased to 50 000. The number of farms in the Sandu bay reached 260 000. This large scale expansion led to frequent outbreaks of low or nil oxygen levels (anoxia), frequent outbreaks of harmful algal blooms (HAB), epidemic fish diseases and mass mortality since then (Zhu and Dong 2013).

Similar problems were also observed in other farming systems and resources like the pearl oyster farms (Fu et al., 2009).

What do we learn from this? Once an ecosystem is damaged and stressed, it cannot be productive. Farmers will have only tales of woe and there will not be any profits. Livelihoods will be affected and can lead to strong social changes including emigration and change of avocation. All these teach us that ecosystem is very important and we have to consider the natural resources and the environmental factors when aquaculture is practiced.

Did you know ?

In the year 2002, for the production of 7.9×10^4 metric tons of shrimp in Bohai Sea and Yellow Sea in China, more than 1.2×10^5 metric tons of uneaten feed was discharged into the sea. (Cao et.al, 2007)

How can aquaculture affect an ecosystem?

In a balanced natural system there is harmony between the food available (plankton, benthos etc.) and the living resources of different trophic levels. These are controlled by several environmental factors like level of nutrients, dissolve oxygen, temperature, salinity, pH, particulate organic matter, total suspended solids and so on.

The benthic systems will have specific sediment texture, organic matter, levels of dissolve oxygen, hydrogen sulphide, pH and so on etc. When the ecosystem is utilized for aquaculture, the services of the living and non-living resources will be affected and this mostly depends on the type of aquaculture system like fed (eg. cage farming) or extractive (eg. bivalve farming) and open (eg. cage farming) or closed (eg. shrimp farming).

Globally, several studies have been conducted to evaluate the Environment impact of farming on the ecosystem. The results of these studies give us an indication on the factor responsible for negative impact and the damage it can cause. Keeping these in mind, aqua farmers are advised to plan their farming activities in such a way that the ecosystem is not stressed and that the farming is productive.

Enhancement of Ecosystem services by aquaculture

Sometimes aquaculture promotes the ecosystem services of the region where it is farmed. Typical examples are that of bivalve culture. The farmed shellfishes remove nitrogen and other nutrients and make it available to in the food chain. They also act as a breeding place for fishes /shellfishes which favour shades and need hard substrates for attaching the eggs. They act as a fish aggregating device. They also serve to reduce the water turbidity to a certain extent.

Ecosystem Approach to Aquaculture (EAA)

In 2006, the FAO Fisheries and Aquaculture Department recognized the need to develop an ecosystem-based management approach to aquaculture to strengthen the implementation of the FAO Code of Conduct for Responsible Fisheries (FAO, 1995). FAO proposed an ecosystem approach to aquaculture (EAA), defined as A strategy for the integration of aquaculture within the wider ecosystem such that it promotes sustainable development, equity, and resilience of interlinked social-ecological systems (FAO, 2010). The strategy is guided by three key principles of which the first principle is related to environment and the ecosystem services and states that Aquaculture development and management should take account of the full range of ecosystem functions and services, and should not threaten the sustained delivery of these to society.

Is EAA significant?

The first principal of EAA states that the ecosystem functions and services should not be affected which means that the services provided by an ecosystem in all aspects such as resource availability and production from other activities depending on the ecosystem (eg fisheries) should not be affected. Generally, natural ecosystems have high resilient capacities. An ecosystem is said to stable when the living resources are able to grow and reproduction thereby maintain the biodiversity of the systems. They are conditioned to the seasonal variation in environmental parameters. Even when the ecosystem is impacted by natural disasters like cyclone or flood, the ecosystem gets back to the original condition after same time. Contrary, to this, when activities like aquaculture are undertaken in an uncontrolled manner in an ecosystem, it can lead to negative impact, which in long term affect the biodiversity and sustenance. This usually happens when the impacts exceed the threshold and limits of the ecosystem.

One typical example is that of bivalve farming. Bivalves feed on the phytoplankton in the surrounding environment where they live. When bivalves are formed in this ecosystem, there is an additional requirement from the farmed bivalves for the phytoplankton available in the area. If the demand for food by biomass of the stocked bivalves in the farm is within the limit available and replenished by the ecosystem within the limited period there is no problem. In case, the demand of phytoplankton exceeds the supply/ regenera-

tion then the food available to the farmed bivalves and the naturally occurring bivalves will be low. This can lead to low growth rates, affect gonad development and spawning and can affect the production. This will affect not only the bivalve farmers but also the bivalve fishers. This will also lead to a chain of events which can affect the nutrient level and survival of other higher trophic resources. To avoid such instances, we have to consider the carrying capacity

What is carrying capacity?

Carrying capacity (CC) is an important concept in ecosystem based management. Earlier, while estimating the CC, only the resource which was farmed was taken into consideration and accordingly CC was defined as the maximum standing stock that may be kept within a particular ecosystem to maximise production without negatively affecting growth rate (Carver and Mallet 1990). Later considering the negative impacts aquaculture can have on the ecosystem services CC was redefined and now CC can be defined as "the amount of change that a process or variable may suffer within a particular ecosystem, without driving the structure and function of the ecosystem beyond certain acceptable limits" (Duarte et al. 2003).

In most aquaculture management programmes, the concept put forth by McKindsey et al. (2006) is considered. Here four different types of CC are considered i) physical ii) production iii) ecological and iv) social. These can be described as given below.

- Physical carrying capacity is the total area of marine or brackish water farms that can be accommodated in the available physical space
- Production carrying capacity is the stocking density of bivalves at which harvests are maximized
- Ecological carrying capacity is the stocking or farm density which causes unacceptable ecological impacts
- Social carrying capacity is the level of farm development that causes unacceptable social impacts



Implementation of carrying capacity concepts

For sustainability, identification of critical limits (i.e. performance standards or thresholds) at which the levels of aquaculture developments can disrupt an ecosystem, thus requiring management actions should be known. These indicators are known as environmental quality standards (EQSs) and are used by planners. The Association of Southeast Asian Nations has also started the process of standardizing water quality standards within the Southeast Asian region. In many countries, an EIA is essential as part of the licensing process for farms over a threshold size. In some regions if the farmer plans to expand an existing site beyond the approved license size then also EIA is required.

The EIA may be defined as “The process of identifying, predicting, evaluating and mitigating the biophysical, social, and other relevant effects of development proposals prior to major decisions being taken and commitments made” (FAO, 2009). The EIA most often provides the framework for the implementation of environmental carrying capacity criteria, although it can also include social and economic impacts.

In Asia, aquaculture farm size is usually small and the EIA may not be worth monitoring individually. However, when many such farms exist in an estuary, there is a need to evaluate the overall impact on the ecosystem which is called strategic environmental assessment (SEIA). This is to ensure that the sum of the small farms will not exceed the ecological carrying capacity. However, such evaluations are rarely done.

For large farms sharing a common water body, like that of shrimp farming in coastal zones, the combined effects of farms on the receiving water body (e.g. a mangrove estuary) is normally not assessed or monitored. However, the combined farm nutrient loads can exceed the ecological carrying capacity. In such situations cluster management is advised.

Cluster management in simple terms can be defined as collective planning, decision-making and implementation of crop activities by a group of farmers in a cluster (defined geographical area for example sharing common water source) through a participatory approach in order to ad-

dress the common risk factors and accomplish a common goal (Ross et al., 2013).

Environmental impacts of different farming systems

Usually coastal aquaculture farms are located in estuaries, where tidal flushing is significant and can play a critical role in determining the carrying capacity and lowering the impact on the ecosystem. A well-flushed estuary or bay can make aquaculture more sustainable, or have a larger carrying capacity, than poorly flushed basins.

Mussels, oysters, scallops, pearl oysters and seaweeds are cultured using racks, rafts or long lines. These farming practices are considered as environment friendly due to their nutrient assimilating capacity and there is practically no feed input required. However, the bivalves can cause localized bio-deposition of pseudofaeces. Since these are concentrates of phytoplankton, they can increase the soil productivity. Though mussels or oysters act as a bio-filter, organic pollution from large-scale mussel or oyster culture in form of pseudofaeces cannot be neglected.

Did you know?

- An individual mussel produces 5.7 mg organic matter per day (Dankers and Zuidema, 1995).
- A typical oyster rack with 420 000 oysters can generate 16 tonnes of faecal and pseudofaecal material during a nine month culture period. (Nunes and Parsons, 1998).

A brief summary of the impacts of extractive type of farming such as bivalve farming on the ecosystem are given below.

- Reduction in phytoplankton / seston
- Increased water clarity leading growth of sea grasses
- Increased abundance of cyanobacteria under bivalve farms
- Higher organic nitrogen, total nitrogen, chlorophyll, phaeopigments in the surface sediments
- Increased sedimentation
- Alteration of sediment texture /sediment geochemistry

- Altered soil Eh
- Lower species diversity in sediment communities
- Reduced macrofaunal biomass
- Modification of current patterns
- Higher abundance of benthic predator communities
- Higher sulphide levels
- Low oxygen levels
- Altered sediment phosphate fluxes
- Deposition of dead bivalve shells

Finfishes and shrimps have to be provided supplementary feed when they are stocked in cages or in earthen ponds. Most of the farms are located in near shore coastal waters and the impacts are localized. In these systems the excess feed and the wastes from the farm can cause ecological damages. Some of the significant damages / changes due to fed type of farming is given below

- Increased nutrient levels in water due to supplementary feed
- Changes in phytoplankton community due to varied nutrient levels
- Increased nutrient levels in sediment
- Altered soil redox potential
- Anoxic conditions in the sediment beneath the cage
- Increased bacterial growth in the sediment
- Different sediment texture
- Changes in benthic community structure
- Altered microbial population
- Escape of farmed species and change in natural diversity
- Increased occurrence of disease
- High BOD levels

Need for sustainability in ecosystems

Though Asia is the largest aquaculture industry in the world, there are only very few large-scale aquaculture corporations in this region. Most of the production comes from millions of small-scale farms owned by individual farmers. This makes

ecosystem management and coordination difficult. Since 1990 there has been rapid growth of aquaculture production supported by technical progress such as technology for manufacture of commercial feeds, seed and aquaculture support systems and this has significantly improved the living standards of most aquaculture farmers. This has also caused the immoderate expansion of farming scale (Dong et al., 1998) and over carrying capacity farming has become a common issue in many coastal and inland systems.

Since most aquaculture farms are situated in the rural and suburban area, which are not economically developed as other regions, local government or policy implementers find it difficult to strictly enforce the laws which curtail farming even if it is for the cause of sustainability. Hence rules related to carrying capacity (eg number of farms per unit area) and water quality management (eg. discharge of effluent water from shrimp ponds) can only be partly enforced.

For different aquaculture systems, the best management practices which support sustained production from the farming system and also support ecosystem services of the adjoining water resources are varied. Farmers and planners are advised to adhere to the EIA procedures and restrict activities which will stress the ecosystem.

Eco labeling and certification in aquaculture

Globally sea food consumers became concerned about the quality of the farmed product during the 1990's which is marketed and also about the damage to the ecosystem done through irresponsible farming. This led to the development of concepts such as eco-labeling and organic farming.

Aquaculture certification is a potential market-based tool for mitigating negative environmental impacts and enhancing societal and consumer benefits (FAO, 2012). The Article 9.1.5 of FAO Code of Conduct for Responsible Fisheries (FAO, 1995) prescribes that "States should establish effective procedures specific to aquaculture to undertake appropriate environmental assessment and monitoring with the aim of minimizing adverse ecological changes and related economic and social consequences resulting from water extraction, land use, discharge of effluents, use of

drugs and chemicals, and other aquaculture activities”.

Did you know?

Organic aquaculture was responsible for an estimated US\$46.1 billion internationally in the year 2007. The market for organic aquaculture shows strong growth in Europe, especially France, Germany and the UK - for example, the market in France grew 220% from 2007 to 2008 (Wikipedia)

At present there are at least 30 certification schemes relevant to aquaculture and these include schemes promoted by retailers, aquaculture industry, governments and NGOs; organic certification schemes; fair trade certification schemes and other schemes. The number of certification and eco labeling schemes for aquaculture products has significantly increased over the years.

Organic certification addresses the processes involved in production rather than the qualities of the product itself. Organic farming is based on holistic production management systems which promote and enhance agro-ecosystem health, including biodiversity, biological cycles and biological activity. In general, organically farmed fish which is farmed without using antibiotics and pesticides is perceived to be more “natural” and therefore healthier, or even tastier. Because of these new concepts which promote eco-friendly aquaculture, there is a tendency to prevent environment degradation and promote sustainability.

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Site selection and water quality in mariculture

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Introduction

Mariculture is the cultivation and harvesting (or farming) of fish, shellfish and other aquatic species, including seaweeds, utilising seawater as a growing medium. Site selection and water quality in mariculture is one of the most important factors that determine production and mortality. Choice of site for mariculture is of supreme importance since it affects water quality and influences the economic viability. Many problems affecting the culture as well as the environment can be evaded by proper site selection. Mariculture includes culture of various marine organisms and the present document looks into, site selection and water quality aspects for culture of marine fish, shrimp, bivalves and seaweeds only.

Marine fish

Historically, culture of marine species has been done in situ in coastal waters. Due to increased coastal developments, clean water and suitable sites for coastal aquaculture are lesser. Open sea culture is a major avenue for expansion of culture of marine fish. Offshore culture of marine fish is usually practiced in cages. For cage aquaculture, the site can be in open sea, estuarine or backwater.

Criteria for selecting a site for marine cage culture

Environmental Criteria

Depth

A depth of 6–10 m at low tide may be considered as ideal condition. Cages should be in sufficient depth to maximize the exchange of water, yet keep the cage bottom well above the substrate (sea floor) in order to avoid interaction between the cage bottom and sea floor. Shallow bays with limited depth of water under cages are not favorable for water renewal. It can cause chemical and bacterial interactions, net damage and predation of the fish by crab and bottom organisms.

Wind and waves

The wind can determine the suitability of a particular site or area for cage fish culture through its influence on cage structures and caged stock. Areas of violent storms are to be avoided. But, effects due to moderate winds can be profitable since it helps the mixing of water. Maximum permissible wind velocity limit is 10 knots for floating cage.

Wave size is determined by wind velocity, wind duration and the distance of open, unobstructed water across which the wind blows; and also by the waves present when the wind starts to blow. At the windward end, waves are poorly developed with small wave heights and short periods of oscillation. However, waves develop with distance, reaching maximum size when they attain the same velocity as the wind. Wave height increases with wind velocity and wave energy increases proportionally with square of wave height. The maximum limit of wave height for floating cages is 1 m.

Currents and tide

Good water exchange through cage is essential both for replenishment of oxygen and removal of waste metabolites. A weak and continuous current stream is favorable to bring oxygen and remove wastes in a cage. However excessive currents impose additional dynamic loadings damaging cages, reduce the cage usable volume due to the deformations of the net and may adversely affect fish behavior. The limits for current velocity is with a minimum of 0.05 m S⁻¹ to a maximum of 1 m S⁻¹. In all except a few coastal regions of the world, tidal currents form the predominant source of surface water currents. Attractive forces exerted by the moon and sun on the Earth produce tidal waves. The crest and trough of the wave are termed high and low tide respectively, while the wave height is referred to as the tidal range. Associated with the rise and fall of the tide are the horizontal motions of water or tidal currents. Maximum current velocity occur at the middle of the

rise (flood) and fall (ebb) i.e., during the mid time between highest and lowest tide. For marine cage culture, tidal amplitude of < 1 m is preferred. Current velocity during monsoon is mainly influenced by littoral current, strong winds, wave effects and increased river discharge. Hence there is every chance that current velocity can exceed its permissible maximum limit prescribed for marine cage culture during monsoon. Monsoon season is therefore generally avoided for marine cage culture activity.

Substrate and bottom dynamics

The sea bottom floor ranges from rocky to soft mud. This bottom floor is the cage site substrate. Mud or rock bottom may cause difficulties for a safe and reliable anchorage for cage. A sandy or gravel bottom is generally suitable. It is very important to avoid areas of erosion, transportation or accumulation of oxygen consuming organic material. If the bottom water exchange is small, oxygen deficiency will be higher and this can lead to the formation of hydrogen sulphide (H_2S) which at certain concentrations may become lethal.

Carrying capacity

A major consideration in site selection is carrying capacity of the site, i.e., the maximum level of production that a site might be expected to sustain. Intensive farming results in production of wastes which can stimulate productivity and alter the water quality. Hence the profitability or even viability is affected.

Water Quality Criteria

Temperature and salinity

Fish and other farmed organisms have no means of controlling body temperature, which changes with that of environment. A rise in temperature increases metabolic rate and causes a concomitant increase in oxygen consumption and activity as well as production of ammonia and carbon dioxide. Salinity is a measure of the amount of dissolved solids present in water and is usually expressed in parts per thousand. Its relevance to mariculture lies principally in its control of osmotic pressure, which greatly affects the ionic balance of aquatic animals. Rapidly fluctuating conditions of temperature and salinities are harmful for marine life culture. Considerable seasonal changes also to be taken care of during the culture period.

For most tropical marine life aquaculture, a temperature of 26-28 °C with no abrupt changes is considered as suitable. Preferred salinity range is within 25 – 40 ppt, evading abrupt changes.

Dissolved oxygen (DO)

Dissolved oxygen is required by all higher marine organisms for the production of energy for essential functions such as digestion and assimilation of food, maintenance of osmotic balance and activity. Oxygen requirements vary with species, stage of development, size and also influenced by environmental factors such as temperature. Solubility of oxygen in water declines with increasing temperature and salinity. If the supply of oxygen deviates from the ideal; feeding, food conversion, growth and health can be adversely affected. It is therefore important that good oxygen conditions prevail at a site.

During the day, there is a net production of oxygen, but at night, when photosynthesis stops, the algal community in water becomes a net oxygen consumer. The environmental conditions conducive to blooms usually occur during the warmer months in areas subject to high nutrient influxes. Avoid areas of occasional recurrence of blooms for cage culture. External sources such as sewage discharges and agricultural runoff may be important contributors to blooms. However, a sudden upwelling of nutrient rich water from deeper layers of the water body may also stimulate blooms. Marine sites having good bottom current which disperse settling wastes are desirable. Preferred DO level for marine life culture is > 6 mg l⁻¹.

pH

The pH gives an idea whether the water is acidic (<7) or alkaline (>7). Extremes of pH can damage gill surfaces, leading to death and it affects the toxicity of several common pollutants like ammonia and heavy metals. The pH of sea water usually lies in the range 7.5 – 8.5. The suitable pH for mariculture is from 7.8 to 8.4.

Turbidity / Total suspended solids and Colour / Transparency

Turbidity refers to the decreased ability of water to transmit light. It is caused by suspended particulate matter. The quantity and quality of material suspended in water column at any particular moment is largely determined by water move-

ment, which transports, fractionates and modifies solids. Large, dense particles are more easily settled than small, less dense particles. Suspended solids in a suitable site for net cage culture should not exceed 2 mg l⁻¹. But its effects also depend on the exposure time and current speed. Turbidity and color in water may result from colloidal clay particles, from colloidal or dissolved organic matter or from an abundance of plankton. Secchi disk visibility can be taken as a measure of color / transparency of the water in marine life cage culture. Optimum transparency expressed as Secchi disk visibility for marine culture is < 5 m as yearly mean.

Inorganic nitrogen

The level of ammonia-nitrogen in the water should preferably be less than 0.1 mg l⁻¹. The ammonia nitrogen in water by the decomposition of uneaten food and debris at the bottom, can affect the fish. Normally in coastal area, sewage discharge and industrial pollution are the main sources of higher level of ammonia in seawater. Nitrate (NO₃-N) and nitrite (NO₂-N) also contribute to the inorganic nitrogen. The total inorganic nitrogen desirable for culture is < 0.1 mg l⁻¹.

Total inorganic phosphorus

Phosphorous is a limiting nutrient needed for the growth of all plants - aquatic plants and algae alike. However, excess concentrations of P can result to algal blooms. The total inorganic phosphorus for marine life culture is < 0.015 mg l⁻¹.

COD (Chemical Oxygen Demand)

The COD of water represents the amount of oxygen required to oxidize all the organic matter, both biodegradable and non biodegradable by a strong chemical oxidant. Preferred Chemical Oxygen Demand for mariculture is < 1 mg l⁻¹.

Chlorine

Both free and combined, residual available chlorine are extremely toxic to fish. The measurable concentrations of chlorine in water for mariculture is < 0.02 mg l⁻¹.

Heavy metals

Originates mainly from anthropogenic industrial pollution. Avoid sites near to industries and effluent discharge outlets if any present. The toxicity of heavy metals is related to the dissolved

ionic form of the metal rather than total concentration of the metal. Mercury (Hg) is toxic to both aquatic life and humans. Inorganic form occurs naturally in rocks and soils. It is being transported to the surface water through erosion and weathering. However, higher concentrations can be found in areas near the industries. The most common sources are caustic soda, fossil fuel combustion, paint, pulp and paper, batteries, dental amalgam and bactericides. Mercury remains in its inorganic form (which is less toxic) until the environment becomes favorable, i.e. low pH, low dissolved oxygen, and high organic matter where some of them are converted into methylmercury (the more toxic organic form). Methylmercury tends to accumulate in the fish tissue, thus making the fishes unsafe to eat. The total mercury in water for marine life culture should be < 0.05 mg l⁻¹. Lead (Pb) comes from deposition of exhaust from vehicles in the atmosphere, batteries, waste from lead ore mines, lead smelters and sewage discharge. Its toxicity is dependent on pH level, hardness and alkalinity of the water. The toxic effects on fish is increased at lower pH level, low alkalinity and low solubility in hard water. The lead in water for marine life culture should be < 0.1 mg l⁻¹. Copper (Cu) enters the environment naturally through the weathering and solution of copper minerals and from anthropogenic sources. Anthropogenic sources of copper in the environment include corrosion of brass and Cu pipes by acidic waters, industrial effluents and fallout, sewage effluents, and the use of Cu compounds such as CuSO₄ as aquatic algicides. Major industrial sources of copper include smelting and refining industries, copper wire mills, electroplating, metal finishing, coal burning, and iron and steel producing industries. Large quantities of Cu can enter surface waters, particularly acidic mine drainage waters, as a result of metallurgical processes and mining operations. The toxicity of Cu to marine organisms in marine and estuarine environments is influenced by physical factors and chemical characteristics of the marine environment. The copper in water for marine life culture should be < 0.02 mg l⁻¹.

Pesticides

Pesticide refers to any chemical used to control unwanted non-pathogenic organisms, including insecticides, acaricides, herbicides, fungicides, algicides and rotenone (used in killing unwanted

fish) (Svobodova, 1993). These chemicals are designed to be toxic and persistent, thus it is also of concern in aquaculture. It can affect the quality of the aquaculture product as well as the health of the fish and humans. Pesticide can be split into seven main categories namely, inorganic, organo-phosphorous, carbamates, derivatives of phenoxy-acetic acid, urea, pyridinium, and derivatives of triazine (Dojlido and Best, 1993). Among these, the chlorinated form is of particular concern due to its persistence and tendency to bioaccumulate in fish and shellfish. Some examples are dichloro-diphenyl-trichloro-ethane (DDT), aldrin, dieldrin, heptachlor, and chlordane. The most common sources are agricultural run-offs, effluents from pesticide industries and aquaculture farms. The safe level of DDT group in water for marine life culture should be $< 0.025 \mu\text{g l}^{-1}$.

Accessibility: The culture site should be near a shore preferably with a jetty for boat connection with farms and near a good road for land transportation.

3. Shrimp

Environmental Criteria

Shrimp farms should not be located in Mangrove forests. Shrimp farms should not be located in ecologically sensitive areas like marine parks, sanctuaries etc. The nearness of shrimp farms to other land uses may have some negative impacts due to the seepage of water, which will increase the salinisation of land and water resources. So buffer zones should be provided in such areas depending on the soil conditions. Sandy and/porous soils should be avoided. Shrimp farms should not be located on natural flood drains. Water spread area of a farm should not exceed 60% of the total area of the land. Wherever the intake and outfall are in the same creek, overcrowding of the farms should be avoided. The total area of shrimp farms that could be supported by a creek depends on the water flow, tidal amplitude, water retention time, and level of intensification of culture systems. This is defined as the 'carrying capacity' of the particular creek and can be estimated taking all these parameters into account. New farms can be permitted only after an assessment of the carrying capacity of the creek.

Soil quality

Soil is the most important component in a culture system. The quality of soil should be ascer-

tained for pH, permeability, bearing capacity and heavy metal content. Soil with low pH of below 5 and acid-sulfate soils should be avoided. Similarly soils with high concentrations of heavy metals also should be avoided. The soil characteristics suitable for a shrimp culture farm are as follows.

Soil quality	Optimum level
pH	7 – 8
Organic carbon	1.5 - 2.5%
Calcium carbonate	> 5%
Available nitrogen	50 -75 mg/100 g soil
Available phosphorus	4 - 6 mg/100 g soil
Electrical conductivity	> 4 mmhos/cm

Generally clayey loam soils are preferred. Sandy soils are seepage prone and will lead to problems of salinisation of adjoining land and water resources. Further, maintenance of a farm in sandy area needs high capital and operational costs. Hence, sandy areas should be avoided.

Water Quality

Availability of good quality water in required quantities is one of the most important prerequisite for sustainable aquaculture. While locating the farm site, careful study should be made on the source of water, quantity of water available during the different seasons and the quality of water. The optimal levels of various water quality parameters required for the best growth and survival of cultured shrimps are presented below.

Water quality parameters	Optimal level
1. Temperature (C)	28 -33
2. Transparency (cm)	25 -45
3. pH	7.5 – 8.5
4. Dissolved oxygen (ppm)	5 – 7
5. Salinity (ppt)	15 – 25
6. Total alkalinity (ppm)	200
7. Dissolved P (ppm)	
9. Nitrite - N (ppm)	< .01
10. Ammonia - N (ppm)	< .01
11. Cadmium (ppm)	
12. Chromium (ppm)	< .1
13. Copper (ppm)	< 0.025

14. Lead (ppm)	< 0.1
15. Mercury (ppm)	< 0.0001
16. Zinc (ppm)	< 0.1

Redox-potential

Anaerobic condition can be developed in pond, when input of organic matter exceeds the supply of oxygen needed for decomposition of organic matter. This reducing condition can be measured as the redox potential (Eh). Redox potential indicates whether the water or soil is in reduced condition (Eh with '-' ve value) or oxidized (Eh with '+' ve value) condition. Reduced or anaerobic sediments may occur at the pond bottom of heavily with heavy organic load and poor water circulation. Under anaerobic condition of the pond bottom, reduced substances such as H₂S, NH₃, CH₄ etc. are formed which are toxic to benthic organisms. In shrimp ponds, development of highly reducing conditions at the surface of the pond mud is highly undesirable. Water circulation by water exchange, wind or aeration helps to move water across mud surface and prevent the development of reduced condition. Draining at the centre of pond, as is being practiced by some farmers, is an ideal remedy for the prevention of formation of highly reducing condition during the last phase of culture period. Bottoms should be smoothened and sloped to facilitate draining or organic waste and toxic substances. The redox potential (Eh) of mud should not exceed -200 mV. Hydrogen sulfide can severely affect shrimp growth in pond. H₂S is produced by chemical reduction of organic matter that accumulates and forms a thick layer of organic deposits at the bottom. The bottom soil turns black and a rotten smell is discharged if disturbed. High levels of hydrogen sulfide would affect directly demersal or burrowing shrimps such as *P. monodon*. At levels of 0.1–0.2 ppm in the water, the shrimps appear to lose their equilibrium and die instantly at a concentration of 4 ppm. Using iron oxide (70% ferrous oxide) to treat the bottom soil containing high levels of H₂S would not be economical. The cheaper means is by frequent exchange of water to prevent building up of H₂S in the pond.

4. Bivalves

Bivalve mariculture is carried out both in coastal and estuarine waters. The success de-

pends largely on proper site selection. Consideration should be given to primary factors (physical, ecological and biological) and secondary factors (risk, economics and legal) that are critical to the species selected. In addition, the site should be suitable to the method or system intended to be practiced.

Temperature

The ideal water temperature for better growth rate of mussel in farm is 25–33 °C. For edible oyster, the water temperature range is 21–31 °C.

Water depth

The depth of water column of a location determines the type of culture method to be adopted. For mussel culture method, it can be in the range from 1–15 m at average mean low tide. For culture in the estuarine conditions, even 1 m depth is suitable for horizontal culture of mussels in lesser muddy bottom conditions. For edible oyster culture, sheltered areas with a depth ranging from 2 to 5 m offering protection from waves are desirable. In areas where the mean tide level is < 1.5 m, bottom culture on rocks or other materials can be practiced. The most important consideration with regard to water depth is avoiding long exposure periods during the extreme low-tides.

Water current

Bivalve culture sites should not be in the vicinity of strong currents as strong currents usually generate high turbidity and high siltation rates. However, moderate currents (0.17–0.25 m/s at flood tide and 0.25–0.35 m/s at ebb tide) are needed to provide adequate food supply as well as to carry away the excessive buildup of pseudofaeces and silt in the culture area.

Salinity

Mussels grow well above 20 ppt, but the ideal salinity for rearing is 27–35 ppt. Open coastal areas are usually fully saline with minor seasonal variations. In estuarine areas, decrease in salinity is usually the major and frequent problem, mainly caused by the influx of freshwater from rivers or land runoff during the rainy season. Therefore sites with a high inflow of fresh water are not suitable for the farming of mussels. For edible oyster, the preferred salinity range is 22–35 ppt.

Turbidity

The presence of suspended particles above a certain level disrupts the filtering activity of the bivalve, which often remains closed to avoid tissue damage and also due to gill clogging. In addition, low primary productivity is often the case in sites of high turbidity due to the reduced penetration of sunlight in the water column. As a result poor growth results due to reduced feeding time and limited food availability. It is found that water containing a high suspended load of more than 400 mg/l have harmful effect on the grow-out of mussels. The maximum suspended load tolerable level varies according to species. A practical method for determining the turbidity level is the use of Secchi disc. Sites having a disc reading less than 15 cm are usually considered unsuitable for bivalve culture.

Primary productivity and food organisms

Clear seawater with rich plankton production (17- 40mg chlorophyll l-1,) is considered ideal for mussel culture. The presence of suitable micro algal species is usually not a limiting factor; however, problems do arise when the availability of food is limited. The carrying capacity of a body of water, (ie., the biomass of animals that the algae food it contains can support) can be exceeded by overstocking, leading to reduced growth.

Source of Seed

Bivalve culture needs a proximity to spat or seed source, which may affect site selection criteria. However, if it has to be transported from elsewhere, it should be transported to the farm site within a reasonable time and cost. Transportation itself is not only costly, but usually negatively affects the quality of bivalve seed due to stressful conditions. The mussel (*P.viridis*) seed can remain without water for about 24 h and hence offers easy transportability.

Parameters in farm site

Temperature

pH

Salinity

Dissolved oxygen (Saturation

Suspended solids (mg l-1)

Substrate and bottom slope

Substrate composition and stability is a major environmental parameter for selection of site suitable to benthic species such as clams. Substrate composition will determine the suitability of an area for a particular species. Oyster bottom culture is limited to areas where the sea floor is firm enough to support some kind of cultch and where siltation is not excessive. The degree of bottom slope is one factor, when the bivalve species is cultured directly on the substrate. Suitable culture beds should have a moderate seaward slope between 5-15 degrees.

Pollution

The sedentary bivalve fauna are exposed to very high probability of contamination and could act as vectors due to their peculiar feeding habits and bioaccumulation potential. Bivalves are known to accumulate trace metals and pollutants. Waters with heavy industrial contamination such as trace metals and organic compounds are therefore unsuitable for mussel farming. Further, shellfish from contaminated areas are known to accumulate bacteria and viruses that are pathogenic to human beings.

Harmful algal blooms

Another criterion of deciding the suitability of potential culture site is eliminating the threat of Harmful Algal Blooms. Some coastal waters are known for the appearance of sudden blooms of certain phytoplankton capable of producing highly potent toxins that are harmful to marine fauna and any other animal that feed on them. Unfortunately, it is often difficult to predict if any area is prone to be affected by these toxic blooms, however, during the site selection process, an enquiry of the past history of the HAB in the area is necessary.

European Union (EU) standards to be met for export of mussel products

Mandatory level

$\pm 2^{\circ}\text{C}$ from normal sea temperature

7-9

2 - 48 ppt

> 80 %

30

Petroleum hydrocarbons	Should not be deposited in the flesh and larvae.
Organo-halogenated substances	Should not exceed harmful levels in shellfish
Faecal coli forms	< 300 in the shellfish & intervalvular liquid

Heavy Metals in tissue: Maximum permissible residual level (ppm)

1 Mercury	1.0
2 Cadmium	3.0
3 Arsenic	75
4 Lead	1.5
5 Tin	250
6 Nickel	80
7 Chromium	12

Pesticides in tissue: Maximum permissible residual level (ppm)

1 BHC	0.3
2 Aldrin	0.3
3 Dieldrin	0.3
4 Endrin	0.3
5 DDT	5

Antibiotics and other Pharmacologically active substances in tissue: Maximum permissible residual level (ppm)

1 Tetracycline	0.1
2 Oxytetracycline	0.1
3 Trimethoprim	0.05
4 Oxolinic acid	0.3

5. Seaweed

As with other aquaculture systems, selection of a suitable site is critically important for a new seaweed farm. The success of Eucheuma farming does not only depend on farming technology, but also to a large extent on the proper selection of the site. Capture fisheries and ornamental fish collecting activities are harmful to seaweed culture by damaging the culture ground, culture facilities as well as the crop itself. The potential site should be free from such conflicting activities. Accessibility to roads, markets and government services and aquaculture services should be considered.

Ecological criteria

Sheltered area and seed availability

A suitable culture site for seaweed is that which is well protected from tidal waves and

strong winds that come from the open sea or monsoonal weather conditions. A good site should be a lagoon sited between an island or coral reefs that are bare during low tide covering the area to prevent destruction or disturbance of seaweeds planted. The availability of local stocks of the species to be cultured at the site is a good indicator that the ecological conditions of the site are favourable for the growth and development of the species.

Water movement

Water movement is a key factor that controls or influences the growth of seaweed. It plays an important role in preventing an increase in pH, caused by consumption of carbon dioxide, and in supplying nutrient. It is also important in water aeration, and preventing the rise in water tem-

perature. Water movement caused by currents is considered a better form of water motion than wave. It is more predictable and less destructive. In an ordinary site, a current of about 20 cm/sec is considered suitable for seaweed culture.

Indicator species

The presence of wild stocks of seaweed at the site or in nearby areas is not only a good indicator of ecological suitability of the site, but also eliminates the problem of seed acquisition. The presence of benthic coelenterate is also an indicator to support the suitability of the site for *Eucheuma* in terms of good water movement, high level of phosphate, silicate, salinity, dissolved oxygen and high transparency. The abundance of soft corals for instance is an indicator of good water movement.

Sea bed

The substratum provides mechanical support or attachment of the seaweeds. Seaweeds have different types of attachment adapted to various types of substrata. *Eucheuma* prefers sand or sandy loam substratum with a limited amount of other seaweeds. The *Eucheuma* will not grow well on bottoms covered with seagrasses. The unwanted seaweeds might compete for nutrients or cover the farm-raised *Eucheuma* resulting in quick deposition of silt on the stems and branches.

A sea bottom with hard coral formations and coral heads is also not a good site for a seaweed farm. It is difficult to secure the stakes and the area is a good habitat for seaweed predator such as rabbit fish, puffer fish and sea urchin.

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Aeration, filtration and disinfection in aquaculture

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Aeration

Among various water quality parameters for a successful aquaculture practice, Dissolved oxygen content in the water is one of the most important parameters, as the oxygen is a vital for all the organisms living in the water and having an aerobic type of respiration. The purpose of aeration is to increase the concentration of oxygen in the water. In scientific aquaculture practices, this is more critical because, often the rate of consumption of oxygen is much higher than the natural rate of replenishment of oxygen in the water through diffusion from atmosphere and photosynthesis of aquatic plants. Oxygen is one environmental parameter that exerts a tremendous effect on growth and production through its direct effect on feed consumption and metabolism and its indirect effect on environmental conditions. Oxygen affects the solubility and availability of many nutrients. Low levels of dissolved oxygen can cause changes in oxidation state of substances from the oxidized to the reduced form. Lack of dissolved oxygen can be directly harmful to culture organisms or cause a substantial increase in the level of toxic metabolites. It is therefore important to continuously maintain dissolved oxygen at optimum levels of above 3.5 ppm.

Typically, dissolved oxygen is measured either in mg per litre (mg/l) or parts per million (ppm) with 0 ppm representing total oxygen depletion and 15 ppm representing the maximum or saturation. The solubility of oxygen is influenced by several factors. The solubility of oxygen decreases as the temperature increases; decreases exponentially with increase in salinity; decreases with lower atmospheric pressure and higher humidity and increases with depth. Water temperature is the main limiting factor in aquaculture farms. Oxygen from atmosphere enters the water naturally through air/water interface. This process is enhanced by wind action which creates a mild turbulence at the water surface.

Need for artificial aeration in culture

system

- Rise in atmospheric temperature causes an increase in the rate of biological degradation of organic matter and subsequent depletion of oxygen concentration in water.
- Prolonged cloudy conditions causes reduction in the photosynthetic activity by green plants in ponds results in reduction in oxygen concentration
- Increased stocking rate of animals in semi intensive and intensive farming practices requires greater amount of oxygen for respiration for all aquatic organisms results in depletion of oxygen concentration in water.
- Aeration in ponds helps in mixing and circulation of pond water. Mixing and circulation is more critical in scientific farming which also helps in feed distribution and waste disposal.

Aerators

Aerators are mechanical devices which increases the dissolved oxygen content of the water. Aerators utilizes the energy input to increases the surface area of water available for oxygen transfer and mix water with oxygen to ensure the liquid medium with of oxygen concentration is brought in contact with oxygen or air.

Oxygen Transfer Process

Three steps are involved in the transfer process of oxygen into water

- a. Transfer of oxygen in the gas to gas liquid interface
- b. Transfer across the gas liquid interface
- c. Transfer of oxygen away from the interface into the liquid

Types of aerators

Different types of aerators used in aquaculture are

1. Gravity aerator: In gravity aerators, the water falls under gravity and air is mixed into it from the surrounding atmosphere. They can be made or natural
2. Surface aerator: this type of aerators are commonly used on ponds and can also be used in large tanks, cages etc. They are used to break up or agitate the surface of water so that oxygen transfer takes place. There are different designs of surface type aerators available. Paddle wheel aerators and spray type surface aerators are commercial type surface aerators.
3. Diffuser aerator: Diffuser aerators inject air or oxygen into a body of water in the form of bubbles. Oxygen is transferred from the bubbles to the water by diffusion across the liquid film.
4. Turbine aerator: Turbine aerator consists of a propeller submerged in the water to be aerated. Circulation of water by Rotation of propeller causes greater aeration to occur at the surface. The main disadvantage of using this type of aerator is that propellers can cause damage to the fishes.

Aeration in aquaculture ponds

Steps to maintain optimum levels of Dissolved oxygen would be to support major factors that increase DO and put into check the factors that decrease DO. Photosynthesis plays a major role in oxygen production; respiration of all living organisms in the pond is the major factor involved in oxygen consumption. Oxygen concentration in pond water exhibits a diurnal pattern, with the maximum occurring during the peak of photosynthesis in the afternoon and the minimum occurring at dawn due to night time respiration. The magnitude of DO fluctuation is small and occurs around the level of saturated DO when plankton density is low and increases as plankton density increases. Supplemental aeration is generally provided during night time when DO increases to levels below 4.0 ppm. Photosynthesis of phytoplankton is the major contributor of DO during the day and diffusion accounts for increases when DO is below saturation at night. Diffusion at night can be tremendously facilitated with the use of aerators, which exposes more water surface to equilibrate with atmospheric oxygen. Through reverse diffusion, an aerator operated during the day will tend to remove supersaturated DO. The

net effect is a milder diurnal fluctuations of DO similar to the conditions of low phytoplankton density. Such conditions are favorable for semi-intensive culture of prawn and shrimp. Photosynthetic oxygen production is also significantly reduced when large scale depletion of plankton population occurs. Under these conditions, flushing out decaying plankton, providing for additional aerators and aerating for additional hours may be necessary to maintain DO at optimum levels. When plankton density is high, the penetration of sunlight through water gets reduced, thereby reducing photosynthetic oxygen production in the bottom of the water column. High plankton density often results from high nutrient loads and under these conditions, large quantities of feed and faecal wastes are found on the pond bottom. This causes an increase in bacterial population and metabolic activity in the bottom sediments leading to high oxygen demand in the bottom sediment. Limited light penetration and increased DO consumption in the bottom may cause significantly lower DO compared to the top layer of the water column. If this causes DO to deplete to lower than critical levels, disastrous effects on the bottom living organisms may happen. Limited light penetration (low secchi disc reading) can also cause differences in the temperature of the top and bottom layer. Temperature stratification usually occurs during calm and warm afternoons.

Filtration

Removal of particles from a water flow is important in aquaculture. Suspended solids, dissolved solids and organic matter were removed from water by filtration of water through suitable media.

Types of filtration

1. Mechanical filtration

In aquaculture, mechanical filtration is used primarily for the separation of solids and liquids. A mechanical filter is a filter that is set into the water flow to collect the particles and larger objects and allow water to pass through. Mechanical filters use differences in particle size of the solution (or mixture) components to extract one part from the other. The simplest type comprises a static screen, a grating or perforated plate. They are usually simple in operation and relatively easy to maintain.

In Sand filters, water is allowed to flow through a layer of sand with particles of varying sizes and depth. The layer is not dense, but contains a number of channels and holes created between the particles that constitute filter medium. When water is passes through the filter medium, particles larger than a certain size will be trapped in the medium.

Various types of mechanical filters are (i) stationary screen (ii) rotary screen (iii) vibratory screen

2. Gravitational filtration

Gravitational filtration utilizes the force of gravity to separate particles from fluid. Density difference of the suspended particles and water is used in this type of filtration. A simple example of gravity filtration is sedimentation. Sedimentation is a process of allowing particulate materials having density greater than that of suspending liquid to settle out under gravitational forces. The settling process of the suspended particles can be increased by aggregating the suspended particles by addition of certain chemicals (coagulation) or by adding chemicals to produce insoluble compounds with suspended particles (precipitation).

3. Biological filtration

Concentration of ammonia in culture water is reduced by biological filtration process. Biological filters are devices to culture microorganism that will perform the given task of reducing the ammonia concentration when water with high ammonia level flows through them. In water both Ammonia (NH_3) and ammonium ion (NH_4^+) are present and their sum is known as Total Ammonia Nitrogen (TAN) and their proportions vary with pH. Ammonia (NH_3) is toxic to fish and their presence in water is important in aquaculture practices.

Biological filters (biofilters) are used to maintain water quality in recirculating or closed aquaculture systems. Biofilters are also used to improve water quality before water is discharged from a facility. Biological filters are formed as a component of the main filtration system which ensures water quality in an aquaculture farm. However it is very important in recirculating aquaculture or aquarium system.

In biological filters, bacteria are used to convert ammonia in various steps. (i) Conversion of

ammonium to Nitrite (ii) conversion of Nitrite to Nitrate and (iii) Conversion of nitrate to molecular nitrogen. The first two steps, known as nitrification, are performed by specific bacteria which oxidize ammonia. The autotrophic bacteria, *Nitrosomonas* bacteria utilize ammonia as a food source and produce nitrite. This nitrite is further converted to nitrate by *Nitrobacter*. These bacteria grow and colonize on the filter medium of biological filter. Both nitrifying bacteria will grow and colonize the biofilter as long as there is food available. The efficiency of the nitrification process depends on the optimum growth of bacteria on the biofilter medium. One of the main factors affecting bacterial growth is the amount ammonia in the water. Other factors regulate are temperature, oxygen concentration, pH, salinity, organic substances and toxic substances.

Disinfection

Water drawn from coastal waters, estuaries and rivers used for various aquaculture activities often forms an efficient means for the introduction and spread of infectious diseases in the system. So it is very essential to have a pathogen-free water source for success in aquaculture. For aquaculture, the supply water to the farm or hatchery is disinfected by various methods. Disinfection of wastewater before discharging is necessary to avoid the pathogen contamination in the environment. Disinfection can be described as the reduction of microorganisms such as bacteria, viruses, fungi and parasites to a desired concentration. The aim of disinfection of water in fish farming is to reduce the risk of transfer of infectious diseases from water to the fish to an acceptable level.

There are several methods for disinfecting water. Disinfectants can be grouped as chemical and non chemical agents. A four group classification for disinfectants is (i) chemical agents (ii) physical agents (iii) mechanical agents and (iv) radiation. Even though various methods can be used for disinfecting water, the quality of the water to be disinfected is of major importance. Pure inlet water is much simpler to disinfect than the outlet water because latter contains more particles. Turbid water and water with a high content of organic substances such as re use water are also more difficult to disinfect and therefore not commonly disinfected. For disinfection of water supplies to aquaculture facilities, UV light and ozone are

commonly used. When starting disinfection, one must be aware of the production of disinfection by products which might be harmful for fishes and humans. Disinfection can be performed in different situations in aquaculture. Water equipment, buildings and effluents can all be disinfected.

Ultraviolet Light

UV light is electromagnetic radiation with a wavelength of 1-40nm located at lower end of the visible spectrum and beyond. The ability of UV light to inactivate and destroy microorganisms varies with both wavelength and the microorganisms to be inactivated. The most effective wavelength for disinfection is 250 – 270nm. UV light damages the genetic materials in the microorganisms which results in their inactivation and death. The efficiency of UV light depends on various factors like lamp intensity, age of the lamp, cleanliness of the lamp surface, distance between the lamp and organisms to be inactivated, duration of UV exposure and purity of water. UV lamps need to be replaced regularly, at least once in a year. UV light transmission through water depends on the turbidity of water, lower in turbid water.

UV lights can be placed in the water flow which is the usual method or above water flow. The dose required to kill pathogenic microorganisms depends on the organism. Most of the common bacteria requires a lower dose while viruses, which are more difficult to disinfect, needs stronger exposure intensity and duration.

Ozone

Ozone is a very strong oxidizing agent, highly toxic to all forms of life. Ozone, is a colourless gas which is very unstable and will quickly be broken down to O₂. Ozone inactivates the microorganisms by damaging cell membranes and nucleic acids, breaking long chain molecules into simpler forms. Another advantageous effect of using Ozone in aquaculture systems is that by oxidation it reduces the amount of NH₃, NO₂, biological oxygen demand. When using ozone as a disinfectant, it is recommended that particles be removed from water before the ozone is added, otherwise much of ozone will be used to oxidize the particles. When adding ozone to water, special injection system has to be used to ensure good gas water mixing. The ozone needs to given sufficient

time to function and oxidize microorganisms. For effective inactivation of pathogens, ozone can be applied in a high dose for a shorter duration and vice versa. Overdosing must be avoided because this may kill the fish. Most pathogens are killed by an ozone dose of 0.1-1.0 mg/L and contact time of 1-10min, but this varies with the organisms.

The water quality parameters like concentration of dissolved organics, particular organics, inorganic ions, pH and temperature will have large impact on the residual ozone concentration after a given time. Because of these variations, it is important to add enough ozone to obtain a satisfactory residual concentration to achieve disinfection.

One of the major problems with ozone disinfection is that it is highly toxic to fishes and humans. Ozone is toxic to fishes even at lower concentrations as it oxidizes gill tissue of fishes. Therefore after disinfection, any residual ozone present in water should be removed or destroyed. An adequate retention time ensures that most of the ozone has reacted and the product is mainly oxygen gas. Being a very strong oxidizing agent, it will oxidize all materials which comes in contact. Ozone will destroy most of the plastic for some extent. Ozone will oxidize metals causing significant corrosion problems.

Chlorine

Chlorine is a very effective disinfectant for water and the most common method used for disinfection of water. It is normally obtained by adding liquid sodium hypochlorite to water, or solid calcium hypochlorite mixed into the water or pure chlorine gas. All these compounds are strong oxidizing agents and have the ability to break down organic molecules.

For effective disinfection using chlorine, specific contact time is required which include time for dissociation in water, time for diffusion through cell wall and time to inactivate selected enzymes. Presence of residual chlorine after disinfection is critical for fishes and overdoing must be avoided. Water containing chlorine is very toxic to fishes. When disinfecting tanks or other equipments with chlorine, it is important that sufficient clean water is used to wash away the chlorine residues produced. There for when using chlorine as a disinfectant, a method for dechlorination must be included.

Marine fish hatchery concept, design and construction

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Introduction

A marine fish hatchery is a complex system consisting various units like seawater intake, water treatment and storage, broodstock holding, indoor algal stock culture, intermediary and outdoor algal culture, rotifer culture, artemia hatching and enrichment, larviculture, nursery rearing, laboratory, feed and chemical storage, aeration facility, waste water treatment and disposal, workshop and staff accommodation area. During the production season proper hatchery management requires specialised skills and total dedication by well-trained personnel. Therefore, proper designing of a fish hatchery will give technical solutions to give best results in terms of convenience, ease of use, effective use of the full production capacity, bio-security, hygienic working conditions and cost effectiveness. Improper designing of the hatchery or construction would result in the risk of ineffective utilization of the facilities, uneconomical operation, increased manpower to manage the facility, chances of cross contamination, loss of stock etc.

Size of the hatchery

While designing the hatchery, the entrepreneur should have a clear idea about its annual production target. This helps to design the hatchery facilities in a proper manner. This decision on the size of the hatchery is a fundamental requirement before commencing the search for suitable sites, technical design and investment plan.

The following issues should be addressed while planning to establish a hatchery:

- fish seeds to be produced (either single or multi species),
- availability of broodstock
- yearly targets as number and size of fingerlings to be produced,
- source of technology and availability of trained manpower

- water quality and availability in different seasons,
- availability of power supply, manpower, motorable roads,
- market demand for the fish seeds

If any of the above aspects is not properly considered during the planning phase, it may result in difficulty in operations and sale of seeds.

Site selection

While selecting the site for marine fish hatchery construction the following are important criteria:

- Motorable road up to the site,
- Stable water quality during most of the months of operation
- Stable temperature during the period of hatchery operation
- Uninterrupted electricity supply
- Away from domestic and industrial waste disposal area,
- Elevation from the mean sea level
- Away from flood prone area
- Protected area from cyclones, hurricanes
- Away from the fresh water canals, rivers and drainages
- Availability of copious volume of fresh water (cleaning & disinfection purpose)
- Away from other shrimp/fish hatcheries

Hatchery layout and design

While designing the hatchery, positioning of various units at suitable places plays important role in easy and economic operation of the hatchery. The water pumping stations and aeration systems should be kept away from the broodstock facility to avoid noise and vibration disturbance to the brooders. Similarly, the packing area has to be placed near to the main entrance, so as to avoid the entry of unauthorized persons and visitors into the bio secured area. Elevation of water storage tanks have to be carefully designed for easy and free gravitational flow of water from these tanks to various units of the hatchery. Every section of the hatchery should have separate entry to avoid cross contamination from one section to other.

Quarantine facility

The quarantine facility is one of the important components of the hatchery, which helps in holding of the broodstock entering into the hatchery to undergo proper treatment and conditioning to avoid the entry of pathogens into the hatchery. The quarantine tanks should be of suitable size to match the size of the brooders to be kept. Facilities for continuous supply of seawater, freshwater and aeration should be provided in this facility. The quarantine facility should be placed either near to the entrance of the hatchery or far off from the production area to avoid cross contamination.

Broodstock holding facility

The broodstock holding facility is the vital unit in the hatchery meant to hold adequate stocks of parent fish to assure a timely production and supply of fertilized eggs of the best quality to the larval rearing unit. Broodstock holding facilities can be located both outdoors and indoors depending of the basic requirement of the brood fishes. Certain brood fishes require strict maintenance of photo

period and temperature regimes; preferably can be located in the indoor area. Whereas, certain brooders may not require such facilities can be reared in outdoors facilities. Generally, outdoor facilities are mainly used for long term holding of immature fishes. In certain cases the outdoor facilities used for quarantining of wild collected parental stock or spent brooders for proper treatment before inducing into the indoor facility

Indoor broodstock holding facilities

The indoor broodstock facility requires a clean environment, with adequate water supply for flow through or recirculation systems. The elevation of water inlet, outlet, drainage canals have to be properly designed for easy and free flow of water. The tank size, water holding capacity and shape has to be designed according to the requirement of broodstock fishes. The broodstock holding tanks have to be painted with suitable coloured epoxy paints to maintain the brooders in a congenial environment and the smooth surface is needed to avoid injury to the brooders and for easy cleaning.



Indoor Broodstock holding tanks with photoperiod control

Broodstock fishes can be retained in the enclosed indoor facilities to avoid exposure to varying environmental fluctuations. Indoor broodstock holding area should have cement/ FRP tanks with proper water supply, aeration lines and lighting to accelerate the gonadal development. When considering installing recirculation systems, enough floor space close to the tanks should be planned in the designing stage to place its various components such as mechanical filters, biological filters, pumps, sterilizers, and heating and photo period control equipments.



Indoor broodstock holding tanks

Outdoor broodstock holding facilities

Outdoor broodstock holding facilities can be located within or nearer to the hatchery premises. Either rectangular earthen ponds or concrete tanks of rectangular/round shape can be constructed between 30 and 100 m³ size, but earthen ponds can be of 500 m³ with proper slope and drainage facilities. These type of facilities are sufficient to hold a good number of fish, but at the same time allows an easy visual control of the captive broodstock. The choice between earthen ponds and concrete tanks is often based on the soil conditions and investment costs and availability of adequate space. While designing the earthen ponds or concrete tanks the space availability, easiness for operation and handling of fishes has to be kept under consideration. In addition, marine fishes can be maintained in the HDPE/ Galvanized Iron Pipe cages in the open sea with proper mooring system. The sea cages can be moored in close vicinity of the hatchery for overall observation, security and safety. While stocking the brood fishes enough care should be taken to feed them with appropriate feed and regular exchange of cage nets of appropriate size.

Spawning and incubation facilities

The tanks where fish are temporarily stocked to obtain fertilised eggs are usually placed in a dedicated area namely spawning facility. These tanks should be located in the quietest location of the hatchery to reduce disturbance to broodstock and adjacent area can be reserved to place tanks for stocking of fertilized eggs for hatching. Dedicated broodstock holding tanks can be construct-

ed adjacent to the spawning tanks to temporarily hold the spent brooders. The spawning tanks should be provided with adequate facilities for altering the photoperiod and temperature regimes, which are essential for faster gonadal maturation. Similarly, the spawning tanks should be provided with adequate water supply and aeration. The spawning area should be maintained clean and bio secured to avoid stress to the brooders. The spawning tanks have to be painted with suitable colour epoxy paints to maintain smooth surface to avoid injury to the brooders and for easy cleaning. Thermal insulated walls and roof are advisable in the areas where temperature fluctuations occur. In such areas additional facilities for controlling the water temperature, heaters with thermostat can be installed. The drains can be placed under the floor and the gutters going to the biological filters should be built well above the floor level to prevent dirt or toxic wastes, such as disinfectants used to wash floors, from entering into the recirculation system.

The spawning tanks are usually round or rectangular (with rounded corners) tanks with water holding capacity ideal for spawning. The spawning tanks can be made of concrete, FRP, or are FRP lined tanks. Tank depth should match the requirement of broodstock fishes and also should be easy to facilitate the work of technicians. Even if automatic egg collectors are used, enough space should be left around the spawning tanks to allow for manual collection of eggs and broodstock handling.

Live feed culture facilities

The live feed culture facility comprises the following units:

- phytoplankton stock culture unit
- rotifer pure strains and small volume culture unit
- phytoplankton starter culture unit
- phytoplankton intermediary culture unit
- phytoplankton outdoor mass culture unit
- rotifer mass culture and enrichment unit
- copepod culture unit
- Artemia nauplii mass production and enrichment unit

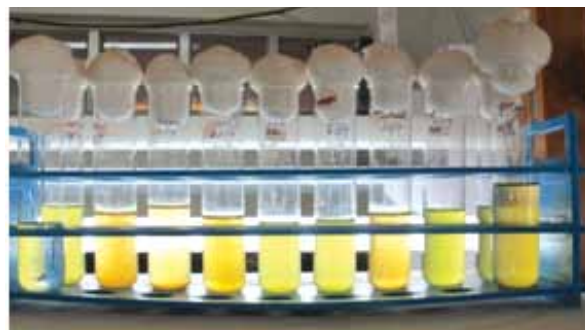
Each unit has to be housed as separate bio secured area to avoid cross contamination. The size and area of the unit has to be designed to match the production requirement. Each unit has to be provided with proper aeration supply, treated seawater and fresh water supply lines, lighting, electricity plug points, proper floor slopping and drainage system.

Phytoplankton stock and starter culture unit

Pure strains of algae as well as starter cultures (from small vessels up to 5-20 litre flasks/carboys), should be kept in an air-conditioned room under sterile conditions to avoid possible contamination. Floor and walls in this unit should be tiled for easy washing and disinfection. An adjacent storage room of smaller size can be constructed to store chemicals, glassware and other consumables. The stock and starter culture facility should have the seawater lines fitted with cartridge filters and UV sterilisers to treat the water prior to use.

The wooden or concrete slabs/ racks should be provide in this unit for placing the stock cultures in test tubes or glass or plastic vessels with adequate lighting system to accelerate the growth. A CO₂ enriched air supply system can be connected to the culture vessels to provide additional source of carbon and to ensure necessary turbulence for uniform mixing of culture media.

Light is very important component in algal culture. The right-size fluorescent tubes have to be conveniently placed to provide light at adequate intensity for pure algal strains and larger starter culture vessels. Aeration is required to create turbulence and to provide oxygen for both pure algae and starter cultures. To avoid the heating effect of the lights installed in the unit, air conditioning is usually necessary to keep the temperature within an optimal range.



Algal Stock Culture



Algal Starter Culture



Algal Starter Culture



Alage cultured in Carboys

Intermediate algae culture unit

In this unit, algae are cultured in large quantities in polyethylene (PE) bags/ Acrylic carboys/ FRP Tanks. They are used directly to as inoculums for culture of larger volumes of algae in outdoor units. The bags/ carboys/FRP tanks have to be housed in a dedicated area adjacent to the stock culture/starter culture unit. The floor of this room should be tiled to facilitate easy cleaning and should have proper slope towards drainage canal.

Adequate fluorescent lights should be provided to accelerate the algal growth. The number of bags/ carboys/FRP tanks required has to be calculated according to the time taken for the culture to mature to the harvestable level and daily requirement for use in the outdoor culture units. At least 20% additional volume has to be reared to supplement the loss due to algal crash and slow growth during different climatic conditions. The aeration, water supply, CO₂ supply has to be provided according to the requirement.



Intermediary Culture of Algae

Outdoor algal mass culture unit

In this unit, algae are cultured in large quantities in FRP/ concrete Tanks. The cement tanks of rectangular size with a holding capacity of 5 -7 tones would be ideal for easy handling. These tanks receive inoculums from the intermediary culture tanks. The FRP /concrete tanks have to be placed in a dedicated area adjacent to the intermediary culture unit. Sufficient lighting arrangement has to be provided through transparent roofing. The tanks should be painted with white epoxy paint for improved light reflection. The floor of this

unit should be tiled to facilitate easy cleaning and should have proper slope towards drainage canal. The number of FRP /cement tanks required has to be calculated according to the daily requirement of algae for the rotifer culture facility and larvi-culture unit. Time taken for the culture to mature to the harvestable level also should be taken into consideration while calculating the requirement. At least 20% additional volume has to be reared to supplement the loss due to algal crash and slow growth during different climatic conditions. The aeration, sea water and fresh water supply, has to be provided according to the requirement.



Outdoor algal culture unit

Rotifer stock and starter culture unit

A similar set of facility like phytoplankton stock and starter culture unit except CO₂ supply shall be provided for the pure culture and starter culture of rotifers. Care should be taken to avoid cross contamination while maintaining different varieties and sizes of rotifers maintained in this unit.

Rotifer culture and enrichment unit

In this unit rotifers are cultured in large quantities in tanks of 1 to 5 tone capacity. This unit has to be little away from the algal culture units to avoid cross contamination. The tanks can be rectangular in shape made of FRP material or cement tanks with Epoxy painting. The epoxy paint colour has to assist in light penetration and easy for observation and cleaning. The rotifer culture unit has to be divided into multiple sub sections according to type and size of the rotifers cultured. To avoid cross contaminations separate water lines, aeration supply and impediments should be provided. Floor and walls should be covered with tiles for frequent washing and disinfection to

maintain hygienic conditions. As harvesting has to be done in the same room, involving large quantities of culture water, an efficient drainage system is required. The volume and number of tanks has to be designed according to the daily requirement of rotifers and its multiplication rate.

The space occupied by rotifer culture unit is determined by the expected maximum daily consumption of rotifers by the larval fish rearing unit. The calculation should therefore take into account:

- the peak daily amount and type of rotifers to be fed to fish larvae,
- the peak daily amount and type of rotifers to be re-used to inoculate new tanks,
- the individual volume and number of the particular type of rotifer mass culture tanks,
- the average density of enriched rotifer at harvest,
- the average number of days to get a mature rotifer culture.



Mass culture of rotifers

Artemia nauplii production and enrichment unit

The production of Artemia larval stages (nauplii and metanauplii) has to be carried out in a separate area, usually adjacent to the rotifer culture unit. Separate seawater and freshwater lines, aeration lines should be provided. The tanks should be made of FRP material with conical bottom for easy harvest of nauplii. As in the other units, the floor and walls should be tiled to help

maintain good hygienic conditions. As harvesting takes place in the same room with tons of culture water being filtered daily, an adequate drainage system is necessary. The daily requirement of nauplii and metanauplii for the larviculture has to be calculated and accordingly the number and size of the tanks have to be calculated. This also shall match with the hatching percentage of the Artemia cysts and incubation time.

Fish larviculture unit

Rearing of various stages of fish larvae in the hatchery is one of the important activity. All other sections like algal culture, rotifer culture are ancillary units to this activity. The fish larvae are very fragile, sensitive and prone for outbreak of diseases. Utmost care should be taken while designing the larviculture unit. This unit shall be separate from all other units and have proper bio security to avoid cross contamination from outside and tanks within the facility. Adequate lighting has to be provided for this unit. The tanks can be of either FRP or concrete materials. Ideally the tanks should be rectangular with epoxy painting. The epoxy colour should assist the larvae for easy prey catching and feeding. Floor and walls should be tiled to secure proper hygienic conditions and to facilitate frequent cleaning. Since at harvest the tanks are emptied, an adequate drainage system is required.

When a recirculation aquaculture system is used, enough floor space close to the larval rearing tanks should be provided to place components such as mechanical and biological filters, pumps, sterilizers and heating/cooling devices.

The basic considerations for the tanks in larviculture unit includes:-

- the larvae should be easily visible throughout the whole water volume;
- the tank bottom should be easily accessible for daily cleaning; white/ yellow/ pale blue colour facilitates for better detection of dirt;
- absence of dead zones to avoid anoxia, ammonia build-up, etc.;
- optimization of the aeration pattern;
- low cost and local availability of building material;
- optimal use of space;
- Simplified design of support systems (water circulation, air supply, power supply, illumination);
- Minimal manpower requirements for their management;
- A large number of smaller tanks offers better protection against disease outbreaks than few large tanks.

Each tank should be provided with an independent inlet and angle at which water enters the tank will depend on tank design and on the age of the fish population. To prevent excessive turbulence, the aeration in fish larval rearing tanks should be very gentle, with a low air flow. Aeration has to be provided by means of one or more fine diffusers placed on the tank bottom. Freshwater with a few delivery points and a wash-basin for cleaning purposes has to be provided.

The larviculture tanks have to be designed according to the water volume necessary for larval rearing based on:

- number of fish larvae to be reared in each tank,
- amount of fingerlings required per production cycle,
- final larval density and average survival in the larval rearing sector,
- final larval density and average survival in the fingerling rearing sector.



Fish Larviculture Tanks

Nursery rearing unit

The nursery rearing unit is essentially to hold the fish larvae which have attained to the weaning stage to inert larval diets. These larvae are expected to metamorphose within a short span of time similar to the shape of adults. Nursery rearing unit requires larger size rearing tanks. Nursery tanks are usually constructed adjacent to the larval rearing unit to facilitate the easy transfer of fish larvae. Either rectangular or circular tanks can be used as nursery rearing tanks. These tanks shall be made of either FRP/ cement with smooth finishing to

avoid bacterial and algal growth on the walls.

The drainage system should be larger than in the larval rearing unit as many times huge volume of water will be used for flow through. The capacity of the tanks can range from 5 to 10 m³. Bigger size tanks will limit the flexibility required for frequent grading of fish fingerlings. Seawater, freshwater, aeration lines should be provided to this facility with adequate lighting. Space requirement has to be calculated according to the production aimed in each cycle. The tank colour should assist the easy cleaning, visibility etc



Ancillary units

1. Pumping station and Air Blower room

The size and capacity of the pumps have to be calculated according to the water requirement. The number of pumps have to be decided according to the yield of water from each bore well. Drawing water from the open sea should be avoided as it carries lots of dirt, organic matter, eggs and larval forms of different organisms, disease causing organisms, parasites ect., For the hatchery operations drawing water from the sub soil through bore wells will help to get pure filtered sea water. The location of pumping station should be easily accessible, to simplify transport of pumps and other equipments. Further, the pumping station should be located as close as possible to the hatchery to facilitate constant surveillance. The seawater bores should be installed in the deepest part of the sea during the low tide, so as to get continuous supply of sea water during the entire operational period. The pumps should be housed in a room to protect the motors and electrical installations from heat, dust and rain



Seawater Intake point



Seawater Pumps

water. Proper fresh water lines should be provided in the pump room for priming of the pumps.

Air blowers have to be placed nearer to the pump house or nearby area. The blower capacity has to be calculated according to the aeration

requirement in each section. A stand by blower has to be installed for use in the event of failure of the main blower. The blower room shall be covered completely to reduce the entry of dirt into the blowers. Similar covering blower room would provide sufficient proof to avoid sound pollution.



Air Blowers



Stand by Air Blower

2. Seawater filtration and water storage

The sea water pumped should be filtered through various types of filtration systems like slow sand filters and rapid sand filters and can be stored in large sumps designed according to the daily requirement of water in the hatchery. This would help to avoid the entry of unwanted micro organisms, disease causing pathogens into the hatchery. Sea water and fresh water sumps have to be constructed to store sufficient quantity of water required for the hatchery use. The seawater sumps should have provision for chlorination and dechlorination of water. The dechlorinated water can be passed through rapid sand filters and cartridge filters. Over head tanks have to be constructed within the hatchery premises to store copious amount of sea water and fresh water for the storage of water for day to day use. These tanks should be placed in an elevated area with proper gravitational flow it can supply water to all sections of the hatchery. The water requirement should be calculated for the daily operations and accordingly

3. Electrical Generator Room

An electrical generator of suitable capacity has to be installed in the hatchery premises after calculating the load requirement for the blowers, pumps, general lighting and heating equipments. The generators have to be installed in a sound proof casing to avoid sound pollution and preferably, they have to be placed far off from the broodstock holding area. In event of failure of electricity supply from the local authorities for a longer period, the captive generators shall have the capacity to support the entire hatchery operations. A stand by generator is needed for pumps and blowers and general lighting in the event of the failure of the main generators.

4. Workshop and Storage Room

A full-fledged workshop is required in the hatchery to meet the day to day maintenance activities like pump repair, electrical maintenance works, blower repair etc., This unit has to be provided with electrical supply, welding machines, cutting tools, pump and blower repair equipments and accessories. This facility should have a storage room for stocking of aeration & water pipes, other utilities of the hatchery.

5. Fish Feed Store

This storage facility should have air conditioning unit to store the larval feeds, inert diets, Artemia cysts, hormones, antibiotics, health management chemicals etc. This store room should be kept away from the area where water is extensively used. Deep freezers can be installed outside this room to store feeds for the broodstock like, squids, crabs, shrimps, fish etc.

6. Laboratory Room

The hatchery laboratory room has to be located close to the phyto/zooplankton unit or larviculture unit. This laboratory will house microscopes, auto claves, Hot air ovens, etc. Its size depends on the type and number of staff working in the laboratory.. The laboratory should be large enough to allow working together in a comfortable way while performing their routine analyses or carrying out tests. Furniture in the laboratory

should be similar to that of a research laboratory, including, anti-corrosion benches for scientific instruments, cupboards with transparent doors for storing glassware and chemical products, and large desks with shelves. Cement floored open areas for drying of glassware, utensils, tanks etc., should be provided in the hatchery premises.

7. Office and seed packing area

Office and seed packing area should be located near the entrance of the hatchery to avoid entry of visitors into the production facilities. A residential accommodation area for the technical staff and kitchen also should be located near the entrance. A parking area for the vehicles should be provided near the entrance of the hatchery to avoid moving of vehicles within the production area. The whole hatchery area should have proper fencing/compound wall to avoid entry of animals and unauthorized persons.

Broodstock development and breeding of marine finfishes

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Introduction

In recent years, mariculture has been growing rapidly on a global basis especially with the development and expansion of sea cage farming. On a global basis, a rapid growth in marine finfish culture is noted. It has increased at an annual average growth rate of 9.3% from 1990 to 2010. Salmonids, amberjacks, sea breams, sea basses, croakers, groupers, drums, mullets, turbot, other flatfishes, snappers, cobia, pompano, cods, puffers and tunas are the major groups which are maricultured. One of the major reasons for the growth of sea cage farming is the availability of breeding techniques that can produce sufficient quantity of seeds of different high value marine finfish. Many countries in the Asia-Pacific Region like Australia, China, Japan, Taiwan, Philippines, Indonesia, Thailand, Malaysia and Vietnam have made substantial progress in the development of commercial level seed production technologies of high value finfish suitable for sea farming. In India, the broodstock development and seed production of sea bass, cobia and silver pompano were developed and standardized for commercial level production.

The major steps involved in marine finfish broodstock development and breeding are the following:

1. Broodstock collection
2. Transportation
3. Quarantine
4. Broodstock development
5. PIT tagging
6. Cannulation
7. Induction of spawning
8. Egg collection
9. Incubation

Broodstock Collection and handling

Broodstock development is the vital and time

consuming procedure in marine finfish seed production. It is not easy to obtain broodstock fish directly from the wild and hence broodstock development is to be done in captivity. The main selection criteria to identify suitable adult fish as broodstock fishes are as follows:

- Body shape, age and colour,
- Absence of deformities,
- Absence of wounds, haemorrhages, infections and parasites,
- Behaviours like quick response to feed and fast swimming

It is advantageous to collect sub-adults for broodstock development. Larger fishes would have crossed the reproductive age and very small fishes will take longer time to sexually mature. In the case of cobia, fish weighing between 8 to 15 kg could be procured while silver pompano could be procured in weight range of 750 gm to 1.5 kg. Stress should always be minimised during capturing and handling of broodstock. It is best to collect broodstock fishes from trap nets, hook & line, etc., as they cause minimum stress to the fishes. Adequate dissolved Oxygen (DO) should be ensured during transportation.

Quarantine

Upon arrival at the hatchery, broodstock fishes are released into the quarantine tanks for prophylactic treatment. Fish anesthetics like MS 222 (50-100 ppm) and Aqui-S (4 ml / 100 L), can be used for broodstock handling. The prophylactic treatment is given to limit the risk of introducing parasites or bacterial diseases into the hatchery facility. Short time exposure of brooders (maximum 5 minutes) in freshwater will help to remove the external parasites. The prophylactic treatment in hatcheries includes a sequence of medicated baths in formalin, malachite green and Oxytetracycline (OTC). Prophylactic treatment can be repeated three to four times within a week. It is preferable to have a flow-through water circulation in quarantine tanks when treatments are not

underway. Smooth inner surface in tanks allow easy and complete cleaning. The following sequence of treatments can be followed:

Day 1: Fresh water bath for 10 minutes and then Oxytetracycline treatment (50 ppm) in seawater for 30 minutes.

Day 2 to Day 7: Treatment with a mix of 200 ppm formalin and 0.2 ppm malachite green for 1-2 minutes, followed by a freshwater dip for 5 minutes. Before returning the fishes to quarantine tanks with filtered seawater, they can be given an Oxytetracycline treatment at 50 ppm for 30 minutes. The fishes should be closely observed during treatments.

During the quarantine, fish should be closely monitored. If the fishes suddenly become immobile or are found with very less opercular movements or are turning upside down, they should be immediately transferred to filtered seawater. The fishes can be fed during the day time when it is not undergoing treatment. Over feeding should be avoided and the fishes can be transferred to maturation tanks after the treatments are over. Apart from quarantine treatment, the broodstock fishes should be given regular prophylactic treatment with freshwater with or without OTC at least once in a month.

Broodstock development

After quarantine, broodstock fishes are moved into Recirculation Aquaculture Systems (RAS) or sea cages for broodstock development. Broodstock development in sea cages was successfully done for cobia at Mandapam Regional Centre of CMFRI. Circular cages of 6 m diameter and 3.5 m depth with HDPE frame were employed for the purpose. The major problem in the development and maintenance of the broodstock in sea cages is the risk of contracting diseases and subsequent loss of broodstock. The sudden loss of broodstock will affect the seed production, since loss of broodstock cannot be made good from the wild immediately. Hence, on shore facilities like RAS is advised for development and maintenance of biosecured broodstock.

The vital aspects which affect development of broodstock are the photoperiod, temperature and broodstock nutrition. In a shore based facility, the photo thermal conditioning can be practiced which will accelerate the gonadal maturation. In

addition, it is also possible to obtain year round spawning in such a controlled system.

Broodstock nutrition

The viability of the larvae is very much dependent on broodstock nutrition. The nutritional components in the diet, the feed intake rate or the feeding period can all affect spawning, egg and larval quality. In the case of tropical fishes, ovarian development is often asynchronous – oocytes in all stages of development are present at the same time and sometimes independent of season. The ovarian development starts with the formation of primary oocytes. During the primary growth phase, the surrounding granulosa and theca cells envelop the oocyte to form the ovarian follicle. In the early stages of secondary growth, cortical alveoli appear and accumulate in the periphery of the oocyte. Even though the oocyte may increase in size several fold during primary and early secondary growth, the most conspicuous size increase occurs during the last part of secondary growth, vitellogenesis. Vitellogenesis is the process of yolk formation and incorporation in the growing oocytes. The yolk protein precursors, vitellogenins, are high molecular weight lipoproteins that are synthesized in the liver and secreted into the blood. The fatty acid composition of the vitellogenins can be affected by long term imbalances in the broodstock diet. It has been well established that feeding broodstock fish with squid, cuttlefish or meals made from cephalopods have beneficial effects. These feed ingredients make the diet more attractive and therefore increase feed intake. Squid and cuttlefish also contain high levels of essential fatty acids.

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

DHA (20:6 \pm 3), which cannot be produced by fish metabolism. Broodstock fishes are fed ad libitum once a day with squids, cuttlefish, crabs, shrimps and chopped oil-sardines depending on the availability.

Tagging

Tagging or physical marking of broodstock fishes through easily detectable methods is very much essential for selection of broodstock for identification, selective breeding and segregation. The most popular method is PIT Tagging. Passive Integrated Transponder (PIT) tag, also known as is a radio frequency device to permanently mark fishes internally. The tag is designed to last the life of the fishes providing a reliable, long term identification method. The PIT tag contains a microprocessor chip and antenna. It has no internal battery, hence the term “passive”, so the microchip remains inactive until read with a reader. The reader sends a low frequency signal to the microchip of the tag providing the power needed to send its unique code back to the reader and therefore fish is positively identified.

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

Design engineers’ calculations suggest that PIT tags can last as long as 75 years or more. There is no battery to fail and the glass encapsulation is impervious to almost everything. PIT tags can be removed or recovered from a primary location and reused indefinitely. Reducing stress to the fish is the prime factor in ensuring the success of the tagging and safety of the fish. Therefore, the fish should be anesthetized during the implantation of PIT tags. Species, size and age should be considered when making a decision about anesthetization and restraint. Sterile implants are advised

but many field conditions do not allow for sterile implants. Equipment can be disinfected prior to use with alcohol and iodine-based solutions. The tag is encased in glass that protects the electronic components and prevents tissue irritation, thereby very much safe to the fish.



Advantages of PIT tag over other tags

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

Disadvantages

- Initial cost is high
- Low detection distance

Procedure of tagging

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

Stepwise protocol

- Use sterile needle or implanter to tag the fish. In field condition, disinfect all the components prior to use with alcohol and iodine-based solutions.
- Read the tag before inserting into the fish and record the identification code or number.
- Catch the fish and anaesthetize it with suitable anaesthetic. In sea cages, it is easier to restrain the fish inside the catching net.
- Disinfect the site of implantation with alcohol or iodine-based solution.
- It is a better practice to keep a standard site of implantation so that the reading will be easier and quicker.
- The tag loaded inside the implanter has to be inserted into the muscle tissues. It is advisable to insert the tag parallel to the muscle fibres to avoid much damage to the tissues.
- The tag should be released slowly and steadily from the implanter while removing the implanter from the tissue in such a way that the tag fills the space created by the implanter needle.
- Once implanter needle is taken out, the site should be disinfected again with alcohol or iodine-based solutions to avoid secondary infection.
- Release the fish as soon as the tagging is over or once it has recovered from anaesthesia.

Cannulation

At the onset of the spawning season, it is necessary to move selected broodstock fishes from maturation tank to spawning tank after assessing the ovarian development through cannulation. Only females with oocytes in the late-vitellogenic stage, with a diameter around 700µm in cobia and 500 µm in pompano, are selected.

Ovarian biopsy can be carried out as follows:

- Female brooders have to be transferred to a small tank containing anaesthesia in sufficient quantity.
- Flexible sterile catheters (1.2 mm internal diameter) can be used for cannulation biopsy.
- Introduce the sterile catheter into the oviduct, up to the ovary for a few cm; then suck carefully a small sample of oocytes up into the catheter and place the sample on a glass slide.
- After sampling, release the animal into the spawning tank, where recovery from sedation will take place.
- Put few drops of filtered sea water on the biopsy sample and examine under the microscope and measure the diameter of the oocytes and record the measurements.



Induction of spawning

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

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



Hormonal administration to cobia

Spawning

The spawning unit should preferably be kept separated from the main hatchery building to avoid disturbance to the spawners and possible risk of disease contamination. However, for economic reasons, it is usual to keep the brooders inside the hatchery in a specific dedicated area. It is preferable to use circular tanks with at least 1.20 m depth. Shape and depth of tanks count for easy and free movement of brooders. Normally the spawning could be noted within 36-48 hours after hormonal induction. The spawning in cobia and pompano takes place normally between late night and early morning hours. The number of eggs spawned by cobia ranges from 0.4 to 2.5 million, whereas, the pompano brooders spawn 0.5 to 1.5 lakh eggs.

Egg harvest

The fertilized eggs of cobia and pompano float and are scooped gently using 500 μ m net. To minimise the presence of poor-quality eggs, which

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

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

Check for the following egg characteristics:

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

Incubation of eggs

It is done in incubation tanks of 3-5 tonne capacity. After hatching, only the hatched fish larvae have to be moved to the larval rearing tanks filled with filtered seawater. Prior to this, the aeration should be stopped briefly to enable the debris and exuviae to settle at the bottom which can

be removed by siphoning. Aeration needs to be adjusted suitably, not too strong to avoid excessive physical collision among eggs, but not too weak either, to keep the eggs suspended in water column. The main purpose of aeration is to prevent clumping and settling down of eggs. Air bubbles should not be too small as seen while using air diffusers instead of stones, as it results in

clumped eggs and damage of the eggs. It is suggested to limit as much the number of air stones as possible. Stocking density can be maintained at a moderate level of 200 to 500 eggs per litre. The development of embryo can be observed at frequent intervals under a stereo / compound binocular microscope. The hatching of eggs takes place from 18 to 24 hours.

Hormonal induction of spawning in marine finfishes

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Introduction

In recent years, especially with the development and expansion of sea cage farming, mariculture is growing rapidly. On a global basis, a rapid growth in marine finfish culture is noted. It has increased at an annual average growth rate of 9.3% from 1990 to 2010. Salmonids, amberjacks, sea breams, sea basses, croakers, groupers, drums, mullets, turbot, other flatfishes, snappers, cobia, pompano, cods, puffers and tunas are the major groups which are maricultured. For most of the cultured species, supply of wild fry from natural sources is insufficient and fluctuates with environmental and climatic conditions. One of the major requirements for the growth of sea cage farming is the availability of breeding techniques that can produce sufficient quantity of seeds of different high value marine finfish. By manipulating environmental and hormonal factors seed can be produced year round rather than relying on wild-collection thereby reducing cost and disease.

Cobia (*Rachycentron canadum*) and silver pompano (*Trachinotus blochii*) are two marine finfish species with very high potential for aquaculture in India. Fast growth rate, adaptability for captive breeding, lowest cost of production, good meat quality and high market demand especially for sashimi industry are some of the attributes that makes cobia an excellent species for aquaculture. In recent years, the seed production and farming of cobia is rapidly gaining momentum in many Asian countries. Envisaging the prospects of cobia farming in India, broodstock development was initiated at the Mandapam Regional Centre of Central Marine Fisheries Research Institute in sea cages during 2008 and the first successful induced breeding and seed production was achieved during March 2010. Subsequently, successful captive breeding and larviculture of silver pompano were achieved during July 2011.

Gametogenesis and reproductive behaviour

A cascade of events leads to release of mature

gametes from ovaries and testes. Marine fishes produce and release sex cells based on maturity of the individuals, their nutrition and overall health, triggered by cues from the environment (temperature, light/dark duration, tides, presence of conspecifics, mates, etc.) that in turn influence their hormonal/endocrine systems. Along with endocrine control there is also a steady, intimate, more sudden interplay of the fishes' nervous system.

Conditioning and triggering of actual spawning involves combining knowledge of modes of reproduction, social factors such as sex ratios, environmental manipulation and possibly direct/exogenous hormonal administration. Either proper environmental stimuli or administration of hormones acting at the level of the hypothalamus, pituitary, or gonads will affect successful release of mature gametes.

Hormonal manipulation

The endocrine system acts like a chemical link between an organism and its environment. Hormones are slow-acting chemical messengers. Along with the faster acting central nervous system they serve to moderate, direct and sustain the physiology of all animals.

Reproduction of fish in captivity can be controlled by environmental manipulations, such as photoperiod, water temperature or spawning substrate. However, the ecobiology of some fishes is not well known, or it is impractical or even impossible to simulate the required environmental parameters (i.e., spawning migration, depth, riverine hydraulics, etc.) for natural reproductive performance. Almost all fishes reared in captivity exhibits some form of reproductive dysfunctions. The dysfunctions probably result from the combination of captivity induced stress and the lack of the appropriate natural spawning environments. In females there is a failure to undergo final oocyte maturation, ovulation and spawning while in males; there is a reduction of milt quantity and quality. In these instances, use of exogenous hor-

mones is an effective way to induce final oocyte maturation (FOM) and ovulation in females and spermiation in males and produce fertilized eggs. In some fishes, these hormonal manipulations are used only as a management tool to enhance the efficiency of egg production and facilitate hatchery operations, but in others exogenous hormones are the only way to produce fertilized eggs reliably.

Hormonal manipulations of reproductive function in cultured fishes have focused on the use of either exogenous luteinizing hormone (LH) preparations that act directly at the level of the gonad, or synthetic agonists of gonadotropin-releasing hormone (GnRHa) that act at the level of the pituitary to induce release of the endogenous LH stores, which, in turn act at the level of the gonad to induce steroidogenesis and the process of FOM and spermiation. After hormonal induction of maturation, broodstock should spawn spontaneously in their rearing enclosures.

Effectiveness of hypophysation (injection with pituitary hormones) is dependent on the stage of reproductive development of recipients. Injection of hormones in an unripe adult will not generally induce gametogenesis or ripening of eggs. Determination of spawning-readiness is sometimes associated with color or marking changes and distension of the body. There are chemical assays of body fluids which can also be used as guides of readiness, but these are not as commonly employed as much as simple hand-stripping of gametes, their mix and microscopic examination as a guide to broodstock fitness.

Care must be exercised in assaying sexual readiness in spawners. Sometimes generally adopted parameters have proven unreliable. An example of this is females with enlarged abdomens, reddish coloration and protrusion of the cloacal region may be due to engorgement of the intestine, or disease, even during the spawning season. It is often necessary to sacrifice some of the broodstock to assess their reproductive stage.

Two methods of injection are in wide practice (1) Intramuscular, in the flank just below the dorsal fin and behind the gill cover. This method is safer but slower working. (2) Intraperitoneal injections are faster acting but involve a greater chance of injury or death as the injections are made into the body cavity.

Maturation and spawning

At the onset of the spawning season, it is necessary to move selected broodstock fishes from maturation tank to spawning tank after assessing the ovarian development through cannulation. Only females with oocytes in the late-vitellogenic stage, with a diameter around 700 μm in cobia and 500 μm in pompano, are selected.

Ovarian biopsy can be carried out as follows:

Female brooders have to be transferred to a small tank containing anaesthesia in sufficient quantity.

Flexible sterile catheters (1.2 mm internal diameter) can be used for cannulation biopsy.

Introduce the sterile catheter into the oviduct, up to the ovary for a few cm; then suck carefully a small sample of oocytes up into the catheter and place the sample on a glass slide.

After sampling, release the animal into the spawning tank, where recovery from sedation will take place.

Put few drops of filtered sea water on the biopsy sample and examine under the microscope and measure the diameter of the oocytes and record the measurements.

Induced spawning

Induced breeding is commonly practiced in most commercial hatcheries. The hormonal treatment is intended to trigger the last phases in egg maturation, i.e. a strong egg hydration followed by their release. However, if eggs have not reached the late-vitellogenic (or post-vitellogenic) stage, the treatment does not work; hence ovarian biopsy is essential for assessing the ovarian development. The human chorionic gonadotropin (hCG) is used at a dosage of 500 IU per kg of body weight in cobia females and 250 IU per kg body weight for males, whereas, for pompano 350 IU per kg body weight is used for both male and female. This dosage can be administered as a single dose on the dorsal muscles. Use of hCG treatment sometimes gives serious setbacks like not all females respond to it, egg quality may be below acceptable standards with hatching rate lower than 80%, being a large molecule it may provoke immunization reaction, and as a result, fish treated with hCG may not respond when treated repeat-

edly with this hormone. However, hCG can be successfully replaced by an analogue of the luteinizing hormone-releasing hormone [LH-RHa des-Gly10 (D-Ala6) LH-RH ethylamide, acetate salt]. It is a small molecule with 10 peptides and acts on the pituitary gland to induce the release of gonadotropins which, in turn, act on the gonads.

Almost 100% of injected fish spawn eggs whose quality usually matches that of natural spawning.

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

Recirculating aquaculture systems

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Introduction

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

Biosecurity

Hatcheries with RAS facility are often fully closed and entirely controlled, making them mostly biosecure - diseases and parasites cannot

often get in. Biosecurity means RAS can continuously operate without any chemicals, drugs or antibiotics. Water supply is a regular route of pathogen entry, so RAS water is often first disinfected or the water is obtained from a source that does not contain fish or invertebrates that could be pathogen carriers.

Water quality and waste management

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

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

Fish tanks

The round or octagonal or square design with rounded corners and the arrangement of in- and outlets of water treatment units support the circular water flow. Additional circular water flow and aeration can be enhanced by aqua jets. The circular flow promotes the behavior of fish. Circular tanks are good culture vessels because they provide virtually complete mixing and a uniform culture environment. When properly designed, circular tanks are essentially self-cleaning. This minimizes the labor costs associated with tank cleaning. Typically, water is introduced into a circular tank at the side and is directed tangential to the tank wall. The incoming water imparts its momentum to the mass of water in the tank, generat-

ing a circular flow pattern. The water in the tank spins around the center drain, following an inward spiral to the center of the tank. Centrifugal forces and the inward, spiraling flow patterns transport solid wastes to the center drain area where they are removed easily. Once the mass of water in the tank is set into motion, very little energy is required to maintain its velocity. The momentum of the water circling the center drain helps sustain the circular flow. The primary disadvantage of circular tanks is that they do not use space efficiently. A circular tank of a given diameter will have about 21% less bottom culture area than a square tank whose sides are the same length as the diameter of the circular tank. This means that if circular tanks are used there will be 21% loss of potential production in a given amount of space.

Aeration systems

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

Carbon Dioxide (CO₂) Control and Removal

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

Stocking number and density

In evaluating RAS production capabilities, the unit most often used is maximum tank or system stocking density (kg/m³ or lbs./gallon). However, in terms of production potential, this unit of measure is meaningless. Fish can be held at very high

stocking densities while feeding only enough to maintain their basic needs. Underfed fish consume less oxygen and produce less waste. Therefore, the stocking rate of a system (fish/m³) and ultimate maximum fish density (kg / m³) achieved within a tank should be defined by the maximum feed rate (kg feed / hr or day) that the system can accommodate without wasting feed and still maintain good water quality. This maximum feed rate capacity will be a function of the water treatment system's design, type of fish being grown, and type of feed.

Solid removal in recirculation systems

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

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

Some type of filters used for the solid wastes

are drum filters, bead filters, screen filters and rapid sand filters.

Biofiltration

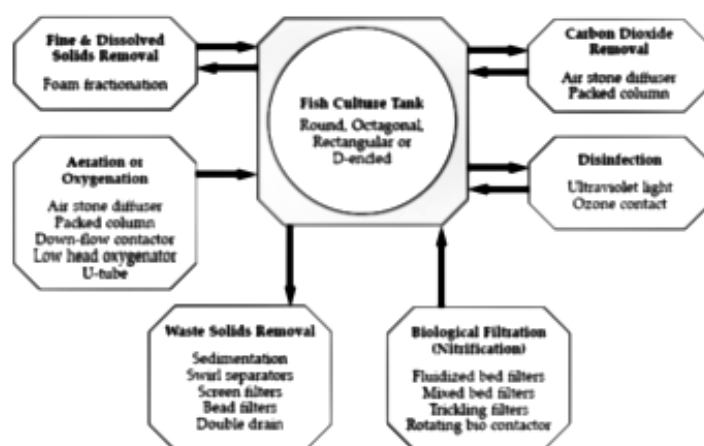
In closed aquaculture systems the accumulation of nitrogen compounds, as ammonia and nitrite, has a deleterious impact on water quality and fish growth. The biological filtration (BOD removal and nitrification) is a fundamental water treatment process in every recycling method for the cultivation of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

Even if nitrate is usually mentioned as the least toxic form in comparison to ammonia and nitrite,

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

Foam fractionation

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



Disinfection of culture water

Installation of suitable UV sterilizers or ozonisers in the water flow would remove unwanted

bacteria, algae and pathogens. The capacity and the flow rate of the UV sterilizer/ ozoniser should be calculated based the on quantity of water to be treated and effectiveness of treatment.



Hormonal administration to cobia



Hormonal administration to cobia



Hormonal administration to cobia



Hormonal administration to cobia



Hormonal administration to cobia



Hormonal administration to cobia

Live feed culture and larval rearing of marine finfishes

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Introduction

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

Live feed culture

Live feeds are the main items in the diet of cultured fish larvae and they are of particular importance when rearing marine fish larvae of the altricial type. The low digestive capacity of the altricial larvae might not be the only aspect responsible for them requiring live feeds. Live preys are able to swim in the water column and are thus constantly available to the larvae. Most formulated diets tend to aggregate on the water surface or sink within a few minutes to the bottom and are thus normally less available to the larvae than live feeds. In addition, since larvae are visual

feeders adapted to attack moving prey in nature, the movement of live feed in the water is likely to stimulate larval feeding responses. Live prey with a thin exoskeleton and high water content may be more palatable to the larvae once taken into mouth, compared with the hard, dry formulated diet. The availability of appropriate live feed is the prime requisite for the successful larviculture of marine finfishes. The chief live feeds employed are (a) rotifers (b) copepods (c) *Artemia* nauplii. Microalgae form the basic requirement for live feed culture and hence microalgal culture is the first step in live feed production.

Microalgae

Microalgae constitute the first link in the oceanic food chain. Nearly 16 genera of microalgae are commonly employed for aquaculture purposes. They are generally free living, pelagic and in the nanoplankton range (2-20 μ m). In aquaculture, microalgae are produced as a direct food source for various filter feeding larval stages of organisms. They are also used as a direct food source in the production of rotifers, *Artemia* and copepods which in turn are used as food for the carnivorous larvae of many of the marine fish species. For rearing marine fish larvae according to the 'green water technique' microalgae are used directly in the larval tanks. This technique is nowadays a normal procedure in marine larviculture and is reported to improve fish larval growth, survival and feed ingestion. The role of microalgae in the rearing water is attributed to (i) providing of nutrients directly to the larvae (ii) contributing to the preservation of live prey nutritional quality (iii) promoting changes in the visual contrast of the medium and its chemical composition and (iv) playing an important role in the microflora diversification of larval gut.

Growth dynamics

A basic understanding of the algal growth dynamics is necessary to carry out their culture. An algal culture goes through the following phases

Lag or induction phase in which there is no increase in cell numbers

Exponential phase in which cell multiplication is rapid.

Declining Phase in which the growth and multiplication of cells will be arrested and slowly the cells show the symptom of decline.

Stationary Phase in which the culture will be stationary without any further cell division for a few days. In the stationary phase if the cells get a new environment, they may start further growth and reproduction.

Death Phase in which the cells will lose its viability and start dying. At this stage the culture will become useless either for re-culturing or for feeding.

Culture methods

The following are the steps involved in micro algal culture

- i. Preparation of culture media
- ii. Identification and isolation of the required species
- iii. Stock and working culture maintenance
- iv. Mass culture

Preparation of Media: Culture media mostly consists of nitrates and phosphates in the ratio 10 : 1 (N : P) besides trace metals and vitamins. Silicate is essential for culturing diatoms, as they have siliceous cell walls. The composition of the two commonly used media viz. Miquel's medium and Conway or Walne's medium is given below:

Miquel's Medium

A. Potassium nitrate - 20.2 g

Distilled water - 100ml

B. Sodium orthophosphate - 4 g

Calcium chloride - 2 g

Ferric chloride - 2 g

Hydrochloric acid - 2 ml

Distilled water - 100 ml

0.55 ml of A and 0.50ml of B are added to one litre of filtered and sterilized seawater.

Conway or Walne's Medium

A. Potassium nitrate - 100 g

Sodium orthophosphate - 20g

EDTA (Na) - 45 g

Boric acid - 33.4 g

Ferric Chloride - 1.3 g

Manganese chloride - 0.36 g

Distilled water - 1 litre

B. Zinc chloride - 4.2 g

Cobalt chloride - 4 g

Copper sulphate - 4 g

Ammonium molybdate - 1.8 g

Distilled water - 1 litre

C. Vitamin B1 (Thiamin) - 200 mg in 100 ml distilled water

Vitamin B12 (Cyanocobalamine) - 10 mg in 100 ml distilled water

Prepare A, B and C in different reagent bottles. Add 1ml of A, 0.5ml of B and 0.1ml of C to one litre of filtered and sterilized seawater.

Equipments and Glasswares: For identification of microalgae as well as for the determination of cell concentration of the culture, a powerful microscope is necessary. For stock culture maintenance the glasswares required are micropipettes, droppers, reagent bottles, culture tubes, conical flasks, Haufkin culture flasks, haemocytometer etc. For mass culture 10 litre polythene bags, 20 litre glass carboys, 100 litre Perspex tanks and 250 litre cylindrical transparent FRP tanks are used for the indoor culture while 250 litre, 500 litre and one tonne fiberglass tanks and 5 tonne concrete tanks are used for the outdoor culture of micro algae.

Isolation of algal species: Twenty litres of water is collected from the water body and enriched with nutrients and left under light until algal bloom occurs. The nutrient added for enrichment should be appropriate to the species required to be isolated. The isolation of a single algal cell from the bloom can be accomplished by any one of the following methods:

1.Simple capillary pipette isolation Method:

The mixed plankton sample is kept in a petridish under a binocular microscope. The desired species is isolated using a capillary pipette and transferred to culture tubes having suitable sterile culture medium.

2. Centrifuging method: By repeated centrifuging the water samples and then by inoculating the deposits, we can isolate several microalgae.

3. Serial dilution Method: This method is used mainly for the isolation of phytoflagellates(i.e. motile species). This involves systematic dilution of the inoculum in five stages (1, 10-1, 10-2, 10-3, 10-4 or 4 steps 0.001, 0.01, 0.1 and 1ml) so that the subject species is well separated from any contaminant. The species thus isolated is transferred to the culture tubes.

4. Agar plating Method: Agar medium is prepared by adding 1.5 gm of agar to one litre of suitable culture medium. This agar medium is sterilized in an autoclave for fifteen minutes under 120 lbs pressure and 100°C temperature. Now the medium is poured in sterilized 15 cm petri dishes and kept for 24 hrs. The required species can be picked by platinum needle or loop under microscope and streaked on the surface of agar plate. After inoculation, these petridishes are placed in an incubation chamber for 7-8 days providing light (1000 lux) and constant temperature (25°C). Within this time , the required species , if it has grown into a colony is removed by platinum loop under microscope and transferred to culture tubes. Further from the culture tubes to small conical flasks and larger flasks, the algae can be grown on a mass scale.

Stock culture Maintenance: The pure culture (0.1ml) isolated from the mixed culture is inoculated into 20 ml culture tubes or 50 ml culture flasks filled with enriched water and incubated in light intensity of 1000 lux (2 tube lights) with photoperiod of 12,hours to produce one million cells/ml. This forms the stock or starter culture for mass culture and thus can be maintained for 15 days. The above procedure should be repeated every 15 days in order to maintain the vigour of the culture.

Working culture maintenance: Some of the 50 ml flasks containing the starter culture are used for inoculating 250 ml flasks. After two days, culture in 250 ml flasks are transferred to 2 litre flasks with enriched water and incubated in light (1000 lux) with aeration for two days to get a density of three million cells/ml. This again is inoculated into 20 litre carboys with enriched water to get three million cells/ml density.

Mass culture: Large scale outdoor culture of microalgae required for hatcheries can be carried out economically by enriching with the following ingredients

Ground nut oil cake -250 gm/tonne
Urea - 10 gm/tonne
Superphosphahate - 5 gm/tonne

Soak the groundnut oil cake in water, then thoroughly smash the same to obtain a milky suspension which can be filtered through a cloth to remove larger sediments. The milky filtered suspension along with the inorganic nutrients (urea and superphosphahate) is added to enrich the water. The required inoculum for mass culture is added and kept under sunlight. The two methods of mass culture commonly employed are – batch culture and semicontinuous culture.

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

Semicontinuous culture: Here the microalgae are allowed to grow until a certain cell density is reached. Then it is partially harvested and fresh medium is added. The growth and harvest procedures are repeated several times before the water quality mandates that the tank be drained and cleaned. It involves less labour but is a less reliable method.

Counting of Micro algal cell density: The apparatus used for counting cells is a haemocytometer with an improved Neubauer ruling. Before counting, both the cover slip and chamber must be rinsed clean and dried. The face of the counting chamber is composed of two gridded surfaces separated by canals. The cover slip is placed on the support bars along the canals and a drop of homogeneously mixed algae suspension is delivered from a Pasteur pipette by touching the pipette tip to the edge of the cover slip where it hangs over the V-shaped loading port. Slight pressure will cause the algal suspension to flow evenly across the surface, but not into the canals or on top of the cover slip.

A small drop of 5 to 10% formalin mixed into

the sample is sufficient to immobilize cells for counting. Each half of the haemocytometer contains nine large grids. Only those algal cells which fall within the four large corner grids are counted. Each large corner grid is further subdivided into 16 small squares. Moving systematically back and forth across the squares, a minimum of 200 algal cells are counted in as many grids as necessary. To determine the algal cell density (number of algal cells per milli litre) in the suspension, the number of algal cells counted is divided by the large corner grid area covered, multiplied by 10,000. For example, if 300 algal cells were counted in 1.5 large corner grids (or 24 small squares), the cell density is $300 \text{ algal cells} / 1.5 \text{ corner grids} \times 10,000 = 2 \times 10^6 \text{ cells per ml}$.

Whenever microalgae are used as a direct food source or as an indirect food source in the production of rotifers, *Artemia* or copepods, growth of the animals is usually superior when a mixture of several microalgal species is used. This probably occurs as different species compensate one another for eventual deficiencies in given nutrients. Special care is needed when selecting microalgae for on growing live feeds for marine fish larvae, in order to avoid the nutritional deficiencies of the latter especially in terms of n-3 highly unsaturated fatty acids. Deficiencies in the n-3 PUFA contents of microalgae may cause severe mortalities and quality problems in marine fish larvae. Such deficiencies may also cause reduced fecundity of rotifer and copepod cultures. Microalgae like *Chlorella* sp., *Nannochloropsis* sp., *Tetraselmis* sp., *Dunaliella* sp., and *Pavlova* sp., *Isochrysis* sp. can be used as algal diet for growing the rotifers. The size, nutritive value, proliferation rate and digestibility of the algae are the critical factors for selecting the algae for the use in marine hatchery.

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

Precautions for maintaining culture asepsis and to prevent contamination are very much needed. All fluids and surface that come into contact with the culture must be sterilized. Natural water used for the culture should be free of pollution and stable in quality. A sand filter reduces the sizes of

suspended particles to 10-20 μm , thereby eliminating most of the zooplankton. Additional filtration by cartridges or sieves reduces particle size to 1 μm . Chemical sterilization such as chlorination – dechlorination and by activated carbon can also be practiced. Natural filtered water is enriched by the addition of mineral salts required for photosynthesis, i.e. metabolisable nitrogen, phosphorus and trace elements including iron and silicon for diatoms. A chelating agent EDTA is often added to prevent precipitation of ferric hydroxide. Vitamins such as thiamine or biotin should be added with due caution because of their rapid degradation due to heat. These salts constitute the enrichment media, the most commonly used being f/2 medium and the medium of Conway. Temperature is often controlled between 18 and 25°C, but this should be fine tuned to each species.

Batch cultures are generally run according to production cycles of 3-7 days. Once illuminated tanks have been cleaned and filled with filtered sterilized water, enrichment medium is added and aeration is provided, and an inoculum is introduced. The algal strains are provided in few milliliters of culture in a test tube. Starting from this sample successive volumes of increasing size are inoculated in order to prepare the biomass required to reach inoculum concentration in the production tanks. The cultures obtained in hatcheries seldom exceed a density of $6 \times 10^6 \text{ cells ml}^{-1}$ at the end of 5 days. The costs of producing microalgae in hatcheries include labour (90%) amortisation (6%), energy (3%) and miscellaneous expenses (1%). In Industrial facilities specialized in the production of microalgae and exploiting production system in controlled conditions such as photobioreactors, the cost of production can be reduced considerably.

When phytoplankton was included in larval rearing tanks, the survival, growth and food conversion index of many marine finfish species were better than in clear water condition. The green water technique (larviculture in an endogenous bloom of phytoplankton and rotifers) and the 'pseudo green water technique' (larviculture in a tank supplemented daily with exogenous phytoplankton and rotifers) have much commercial application in marine finfish larviculture. Micro algae can also influence live feed and larval microbiology. It has been found that exudates of some algal species can either enhance or inhibit

the feeding activity of copepods in cultures. These substances are also involved in the settlement of micro flora required in the gut of fish larvae to prevent intestinal opportunistic bacteria from causing disease. Bacteria associated with live feed can be transmitted to larval fish during feeding. As live prey actively ingest bacteria, it is possible to introduce favourable bacteria as probiotic. In the 'green water technique' of larviculture micro algae contribute to maintaining the nutritional quality of live food and also positively influence on the settlement of a healthy intestinal micro flora in fish larvae. Micro algae can also possibly influence the endotrophic stages (egg and pre-larvae) and early exotrophic stages. Micro algal background has an important effect on the timing and intensity of first zooplanktonic feeding. Micro algae also play a role in intestinal transit and gut repletion. Improvement in the survival at first feeding is the main result of larviculture with micro algae. Improvement in growth efficiency during rotifer period is another result of micro algal background in larval tanks. Early enhancement of digestive and assimilative functions improves the survival and growth of fish larvae and favours the transition to exotrophy. The use of micro algae in tanks increases the production of pancreatic and intestinal digestive enzymes and improves the quality of gut flora. Even after the endo-exotrophic phase, micro algae have a positive effect on larviculture and may increase the resistance of larvae to further stressing or adaptive conditions. The indirect effects of micro algae on larvae are mainly related to water quality, luminosity, the bacteriology of water and the quality and accessibility of rotifers. It is thus evident that strategic use of micro algae in hatcheries during the very early life of marine fish improves the success of first feeding, a prerequisite for efficient survival, growth and quality in fish larviculture.

Rotifers

Rotifers have been used as live feed for cultured marine fish, since four decades. It is well known that a continuous, stable and reliable supply of nutritionally adequate rotifers is the key to the larviculture of marine finfish. Rotifers of the species *Brachionus rotundiformis* and *B. plicatilis* are almost indispensable for larval rearing of most marine finfish.

The success of rotifer cultivation is depend-

ent on selecting the most suitable rotifer species or strain for local culture conditions, maintaining water quality in culture tanks and choosing the most appropriate culture technique. Size, the type of reproduction and reproductive rates are species or strain specific. Culture temperatures, salinities, type of food and its quality - all influence the type of reproduction and its rates. Mass production of rotifers is achieved by encouraging rotifers to reproduce asexually, since sexual reproduction results in males and resting eggs. The amount of food that has to be supplied daily to each tank depends on the reproductive rate of rotifers. Usually 1-4 g of baker's yeast is supplied per million rotifers per day.

The optimal range of pH for culturing rotifers is 7.5 – 8.5 and the pH affects the percentage of unionized ammonia in the water. The pH of cultures play an important role since the toxicity of $\text{NH}_3\text{-N}$ is a function of pH, temperature and salinity. The optimal level for ammonia is $< 1 \text{ mg l}^{-1}$ and the acceptable level of ammonia and nitrite levels is $6\text{--}10 \text{ mg l}^{-1}$. Rotifer cultures require aeration and the dissolved oxygen level should be maintained above 4 ppm. Surplus food is one of the major factors for the deterioration of water quality. This can be avoided by dividing the daily food ration into four to six meals a day or by continuous feeding using a peristaltic pump.

Stock cultures of rotifers are maintained for long periods which facilitate their availability to mass culture wherever they are needed. Natural seawater should be filtered through a $1.0 \mu\text{m}$ bag filter and heat sterilized at 120°C at 15psi atmospheric pressure for 30 minutes to avoid fermentation of insoluble precipitates. The cool sterile seawater can be employed for stock culture. Erlenmeyer flasks (100 ml in volume) or 50 ml sterile disposable tubes can be used for culture. Each heat sterilized flask is filled with 10 – 20 ml sterile sea water and 40 – 60 rotifers are added. Usually, a salinity of 30ppt is suitable for most strains. A drop of concentrated algae is added to each culture and the flasks or tubes are incubated at temperatures ranging from $20\text{--}35^\circ\text{C}$. The cultures are fed ad libitum every 2 days with concentrated algae. Cultures are renewed every 7-10 days. Culture of *Nannochloropsis* sp was found to be the most convenient source of food for rotifer cultures. As in the case of microalgae, mass cultures are done by batch, semi continuous and

continuous culture methods.

Evaluating the physiological state of rotifer culture is very important in hatcheries since larval production depends on a predictable and reliable supply of rotifers. Six parameters viz egg ratio, swimming velocity, ingestion rate, viscosity, enzyme activity and diseases are employed for assessing the state of health of rotifer cultures.

The nutritional quality of rotifers is improved by enrichment, in which rotifers are collected or harvested from culture tanks into containers where they are kept at very high densities and incubated for 8 – 20 hours with enrichment dietary components like HUFA. In addition to nutritional enrichment, rotifers can be enriched with antibiotics or with probiotic bacteria. The nutritional value of rotifers depends on their dry weight, caloric value and chemical composition. The number of rotifers consumed by the larvae determines the quantity of food reaching their gut. In red sea bream, the number of rotifers consumed daily increases with size or age of the larva, 55 – 72 rotifers per 3.9 mm length larvae to 4700 per 11.4mm length larva.

Various methods of storing rotifers have been studied. Frozen rotifers are not usually adequate as feed because of leaching of nutrients. Live *B. plicatilis* can be stored at 40°C at relatively high densities for at least one month. Rotifers can be kept at -10°C without feeding or water exchange for about 2 weeks. *B. rotundiformis* strains are less tolerant to 40°C than *B. plicatilis* rotifer strains and the strains known as SS type are most susceptible and showed lowest survival. Amictic eggs of rotifers can be preserved by cryopreservation in liquid nitrogen after they have been impregnated with cryoprotective agents like dimethyl sulfoxide (DMSO). This method ensures full preservation of genetic traits of importance to aquaculture. Cryopreservation is not a suitable method for preservation of large numbers of rotifers for direct use as feed.

Artificially produced rotifer eggs have been tried as an alternative to daily production of rotifers. The production of these eggs can be manipulated by environmental factors, such as salinity, food quality and quantity, rotifer culture density, exchange of culture media and temperature and varies between *B. plicatilis* and *B. rotundiformis*. The cost of producing resting eggs is very high

and therefore not yet been extensively adapted in hatcheries.

It is evident that rotifer cultures will continue to be indispensable in marine finfish hatcheries. Current methodologies of producing and enriching rotifers are meeting the requirements of the industry. The current need to have very small sized rotifers is difficult to achieve, although several super small strains have been found and cultured. Improved methods for predicting the health of cultured rotifers may be useful in preventing culture crashes). Using preserved rotifers may eliminate the dependence on daily production of rotifers. Cheaper methods of resting egg production are another field which requires research attention in future.

Copepods

Copepods are a major component of the natural diet of marine fish larvae. The advantages of copepods over rotifers are that copepods have wide range of body sizes both within and between species. The early stage nauplii and copepodites can be extremely useful as initial prey for species that have very small larvae with small mouth gape at first feeding.

In extensive methods copepods are collected from nature and inoculated into outdoor tanks to mass produce for fish larval rearing. The larvae are then transferred at densities of 0.01 to 0.321-1. Additional prey may be added during the larval rearing when necessary to maintain prey densities in the range of 200 – 500 l-1. By this method from 1986 to 1994 a total of around 2 million juvenile cod was produced. Disadvantages of this system include the inability to control production and thus food levels and predators. Lack of food results in differential growth in fish larvae. Outdoor production of copepods in ponds or large tanks of 350 – 5000m³ is carried out in Europe and Asia for cod, grouper and flatfish. Filtered seawater by using filters of around 20 – 40 µm is generally used in these systems. Phytoplankton bloom can be induced by application of commercial fertilizers. Filtering devices that allow for selective sieving are used to collect primarily nauplii (80 – 250 µm) and copepodite stages (80 – 600 µm) to inoculate the rearing tanks. A mesh size of 400 – 600 µm was used to inoculate outdoor tanks for grouper rearing with copepodites and adult stages 3 days before stocking the

newly hatched fish larvae at densities of 5 m-3. In this system, using wild harvested copepods, an average survival of 3.4% at harvest correspond to an average production of 0.17 grouper *Epinephelus coioides* juveniles m-3. Regular monitoring of densities of live prey in these outdoor systems is important for the successful rearing of marine fish larvae. An advantage of outdoor ponds over the extensive systems that rely on the local production of zooplankton is the possibility of culturing the zooplankton over one generation before using them as food. Moreover, feeding wild plankton directly to the fish increases the risk of infections. Several attempts to mass culture copepods in intensive systems have been undertaken with varying success. Species with relatively short generation at ambient temperatures are best suited for aquaculture purposes. Species inhabiting in coastal environments are normally more tolerant to variations in salinity and temperature and have a wider thermal and salinity tolerance. The most frequently cultured calanoid species belong to the genera found in coastal waters, such as those of genera *Acartia*, *Centropages*, *Eurytemora* and *Temora*. These copepods are small, with relatively short generation time and a wide thermal and salinity tolerance and are easily adaptable to laboratory conditions. Aeration is required to maintain phytoplankton in suspension and to create small turbulence which helps to distribute copepods within the culture tanks. Most calanoids require large volumes and the adult density rarely exceeds 100 per litre. Successful hatch culture of the calanoid *Acartia* sp was achieved in 1000 litre polyethylene tanks, 1.3m in diameter with a conical base. The tanks are emptied after the 8 day hatch cycle and cleaned and a new batch culture was started. Contamination of copepod culture by bacterial blooms, ciliate infection, other copepods or rotifers may pose a problem. In commercial facilities, contamination by rotifers is most likely to cause the collapse of copepod culture, since the rotifers with their higher reproductive rate would quickly out compete the copepods. Hence these cultures should be strictly kept apart.

Ciliates are utilized by copepods and in periods of low phytoplankton concentration constitute the major dietary source. Ciliates are often an indication of overfeeding and if ciliates are noted in cultures it is advisable to empty the culture using a 60 or 80 μ m mesh, which retains the adult co-

pepods, but allows the ciliates to be washed out. Harpacticoid copepods have several advantages for culturing. They include (i) High tolerance to a wide range of environmental conditions. (ii) Ability to feed on a wide range of live or inert diets. (iii) High reproduction capacity. (iv) Relatively short life cycles (v) Ability to be cultured in high densities. (vi) Requirement for surface area rather than volume (vii) Planktonic naupliar stages (viii) Can be used as tank cleaners in rotifer cultures, other copepod culture or larval tanks.

Filtered seawater can be used for harpacticoid culture and most feeds are acceptable to many harpacticoid species. Algae which quickly sediment are also good feed because bacteria colonise these cells, and the mixture of algae and bacteria form a good dietary combination for harpacticoids. Photoperiod influence offspring production and sex ratio. A photoperiod of 12 L / 12 D was shown to be most favourable for offspring production. Many harpacticoids have wide thermal and salinity tolerances. Ciliates and rotifers in the culture tanks compete for food and may lead to crash of copepod culture.

Improved growth, survival and / or rates of normal pigmentation have been documented for several marine fish species fed copepods alone or as a supplement to other traditional live feeds. The improvements in larval growth, survival and normal pigmentation are generally attributed to the levels of DHA, EPA and / or arachidonic acid (ARA) in the diet and in particular to the DHA: EPA ratio in the diet. Copepods which constitute the major diet for marine fish larvae in nature contain high levels of DHA and other PUFA. DHA levels in wild copepods can be more than 10 times higher than in enriched *Artemia*.

The interest in copepod culture as live feed is gaining momentum in recent years for the rearing of altricial larvae. A few of the culture methods developed to date can be adapted in commercial hatcheries. However there is a need to evolve intensive culture methods for copepods in future. It is felt that the future expansion of mariculture especially of marine finfish depends largely on the development production of resting eggs of copepods on commercial scale.

Artemia

Artemia is widely used in the mass culture of

different sea bream species, sea bass species, wolf fish, cod, turbot, halibut, flounder species, milk fish, surgeon and many shrimps, prawn, crabs and lobsters. Nauplii in instar I and II stages are the most widely used forms of *Artemia* in aquaculture. They are the earliest and easiest live feed, being obtained directly from the cysts.

Several factors are critical for the successful hatching of *Artemia* cysts. Optimal hatching conditions are constant temperature, 15-35 ppt salinity, pH around 8.0. Minimum oxygen levels of 2 mg l⁻¹, preferably 5 mg l⁻¹, maximum cyst densities of 2 gl⁻¹, and strong illumination (2000 lux). Best hatching results are achieved in containers with conical bottom, aerated from the bottom. Transparent or translucent containers will facilitate inspection of hatching, especially when harvesting.

Strong illumination (above 2000 lux at the water surface) is essential, at least during the few hours after complete hydration, to trigger the start of embryonic development. It is advisable to keep the hatching tanks indoors and to provide artificial illumination, so as to ensure good standardisation of the hatching process. When hatching large quantity of cyst, bacterial load rapidly develops. Reducing bacterial development during hatching will improve the hygienic status of nauplii and may result in better hatching. It can be achieved through simple disinfection of the cyst using the liquid bleach solution, through decapsulation. Attention should be paid to the selection of *Artemia* cyst batches with good hatching synchrony (less than 7h between hatching of first and last nauplii) and high hatching efficiency (more than 2 lakhs nauplii per gram).

After hatching and before feeding to fish larvae, the nauplii should be separated from the hatching wastes (empty cyst shells, unhatched cyst, debris, microorganism and hatching metabolites). Decapsulation of cysts results in disinfection of the cysts and also eliminates the introduction of cyst shells to culture tanks.

Most marine fish larvae cannot synthesise DHA, EPA or Arachidonic acid from shorter chain precursors and they must be provided in the larval diet, hence *Artemia* is enriched for enhancing the nutritional value for using as a live feed. Although *Artemia* is often an inferior food source for fish larvae compared with wild zooplankton, the

ability to produce any amount of biomass within 24 hrs, and the constant improvement of enrichment products ensure its continued use in marine fish larviculture. It is quite possible that *Artemia* will gradually be replaced by formulated diets; it is obvious that the use of nauplii will continue in hatcheries at least for a few more years.

Larval feeding behavior

After egg hatching, fish larvae go through important changes to reach the juvenile stage, the most evident being a dramatic biomass increase. Feeding success in fish larvae is critical for obtaining the nutrients and the energy necessary for healthy growth and development that allows them to survive to the end of the larval period. Feeding behavior is the result of interaction of complex processes viz. searching, detection, attack, capture, ingestion, digestion and evacuation. Each of them has a specific pattern that changes throughout development. The feeding strategy is related to the specific characteristics of each species. Availability of suitable prey is one of the most determinant biotic factors, but feeding mode and amount of food intake are also influenced by prevailing environmental conditions.

Searching and detecting food

Searching for prey and detecting them depend on the appropriate functioning of some organs and tissues that become progressively available throughout development. From hatching, larvae are progressively aware of different external stimuli that indicate the presence of potential food items. Searching depends basically on swimming capacity, while detection depends largely on sensory organs. Food detection occurs by means of visual, chemical and mechanical stimuli. Olfaction allows detection of distant stimuli, sight allows the identification of objects at medium and relatively short distance, while touch and gestation need very close or direct contact with the source of stimulus. Most marine fish hatch with immature anatomical features. The sensory organs develop quickly during the first days after hatching. Sight allows the larvae to perceive objects that are relatively close. Altricial marine teleosts hatch with undeveloped eyes although the pigment in the retina appears in a few hours or days. This early retina has only one type of photoreceptor that allows vision only under bright light. Double and mosaic cone structures and rod photore-

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

Locomotor capacity

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

Capture and ingestion

Capture success relies not only on development stage and concomitant hunting capacity but also on the availability and accessibility of prey. Once the prey is perceived, the foraging has three possible results: unsuccessful attacks, aborted attacks and successful attacks. After mouth opening, fish larvae need to learn hunting and have to do it quickly. High prey availability and accessibility are crucial for successfully initiating feeding. Prey size and swimming ability are primary factors determining the efficacy with which the prey is caught. The ability to start feeding after mouth opening is typically affected by prey size. During the very early stages with low swimming capacity, encounter opportunity depends on prey density. Mouth gape limits the dimensions of the prey that

can be ingested. Prey/gape ratio determined in different species usually ranges between 25 and 60%. Searching for appropriate prey of adequate size has been a priority for rearing fish larvae. The established prey sequencing is based on rotifers of different sizes and *Artemia* nauplii and meta nauplii. However, there is a need to search for live feeds below 100 micron size for rearing of very small marine fish larvae. Eventhough copepod nauplii can be employed for this purpose, mass scale production of copepod nauplii for large scale larval rearing is a major constraint. Overall, the current commonly used live feeds, *Brachionus* spp. and *Artemia* spp. meet well the feeding behavior of larvae except very small larvae at mouth opening.

Factors affecting larviculture

Since most of the larvae are visual feeders providing the required light affect the larval survival. During the critical period, the density of the live feed and its nutritional qualities determine the percentage of the survival of the larvae. The density of the larvae of the concerned species should also be regulated in the larviculture tanks for getting good survival. When changing from smaller size live feed to larger size, co-feeding with both sizes of live feeds is needed for a few days. Weaning to formulated feed has to be done with great care. First feeding of the day can be done with appropriate size formulated feed. Feeding with live feed can be continued till all the larvae are weaned to formulated feed. Different sizes of formulated feeds need to be used as per the mouth size of the larvae. The marine fish larvae exhibit highly differential growth even from very early stages (in the case of cobia, starting from the first week) and hence grading from an early stage is also very much needed for increasing the survival. In addition, variety of other factors such as tank colour, size of the tank, water temperature, water quality, etc., affect the larval survival and growth. From the foregoing, it is clear that the larviculture of marine finfish is highly complicated, unless each and every factor is taken care of, the survival and growth of the larvae will be very meager.

Microalgal culture and maintenance in marine hatcheries

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Introduction

Unicellular marine microalgae are widely used as food in the hatchery production of fish and shellfish. Molluscs like oysters, mussels and clams filter them from the seawater in all stages of life. Rotifers and brine shrimp also ingest algae, and these are then used as food for fish and prawn larvae. In hatchery systems algae are added to the larval rearing tanks to improve 'quality' of water as green water systems. The production of algae is very critical in successful hatchery management.

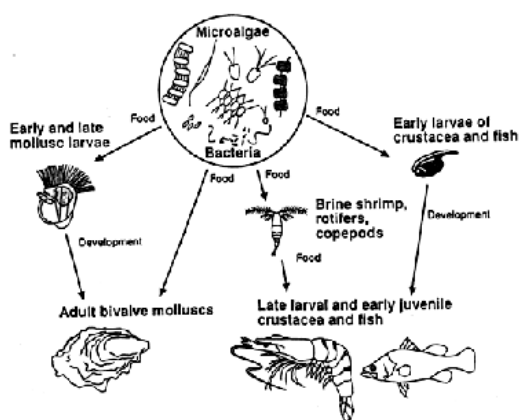


Fig.1 The central role of micro-algae in mariculture (Brown et al., 1989)

Marine algae are single-celled plants and like all plants, contain chlorophyll, which traps the energy from light and uses it to convert nutrients and carbon dioxide dissolved in the sea water into organic matter. Microalgae are the primary producers of the sea. Among microalgae, flagellate and diatom species, are primary producers at the base of the marine food chain. They are cultured in hatcheries in suitably treated seawater enriched with nutrients, which include nitrates, phosphates, essential trace elements, vitamins and carbon dioxide. Synthetic seawater may be used but it is expensive except for small laboratory scale cultures. The culture microalgae arise because the natural phytoplankton content of seawater is insufficient to support growth of high

densities of larvae and juveniles reared. Particularly in the hatchery, the water treatments will remove almost all of the natural phytoplankton which then needs to be replaced from cultures of preferred, high food value species. In this context, few of the naturally occurring algae of good food value are amenable to artificial culture.

Major classes and genera of cultured algal species

The major classes of cultured algae currently used to feed different groups of commercially important aquatic organisms include species of diatoms, flagellated and green algae, and filamentous blue-green algae, ranging in size from a few micrometers to more than 100 μ . 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*, *Pavlova lutheri* and *Chlorella* spp. The basic methods of algal culture have changed little over the years. Hatcheries have either opted for indoor, intensive culture with artificial illumination, usually external to the culture vessels, or outdoor, extensive culture in large tanks or ponds utilizing natural light. The intensive techniques are satisfactory in terms of reliability and productivity but are expensive in terms of capital outlay and labour, while the extensive methods tend to be less reliable and, sometimes not very productive.

Isolation of pure algal strains

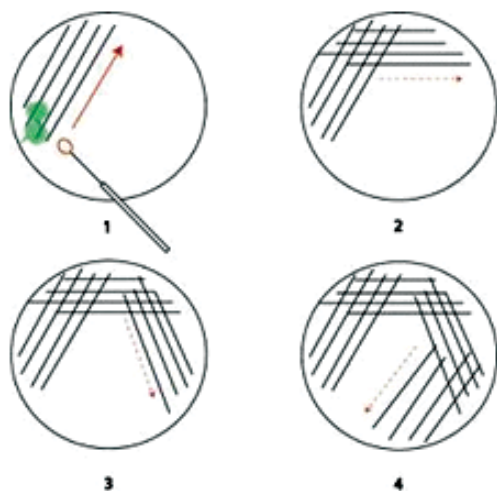
Sterile cultures of micro-algae used for aquaculture purposes may be obtained from specialized culture collections. A list of culture collections is provided by Vonshak (1986) and Smith et al. (1993a). Alternatively, the isolation of endemic strains could be considered because of their ability to grow under the local environmental conditions. Isolation of algal species is not simple because of the small cell size and the association with other epiphytic species. Several laboratory techniques are available for isolating individual cells, such as serial dilution and successive plating on agar media, and separation using capillary pipettes. Bacteria can be eliminated from the phytoplankton culture by washing or plating in the presence of antibiotics. The sterility of the culture can be checked with a test tube containing seawater with 1 g.l⁻¹ bactopeptone. After

sterilization, a drop of the culture to be tested is added and any residual bacteria will turn the bac-topeptone solution turbid. The collection of algal strains should be carefully protected against contamination during handling and poor temperature regulations. To reduce risks, two series of stocks are often retained, one which supplies the starter cultures for the production system and the other which is only subjected to the handling necessary for maintenance. Stock cultures are kept in test tubes at a light intensity of about 1000 lux and a temperature of 16 to 19°C. Constant illumination is suitable for the maintenance of flagellates, but may result in decreased cell size in diatom stock cultures. Stock cultures are maintained for about a month and then transferred to create a new culture line

Agar plating

Agar plating technique can be used to isolate algal strains from raw seawater and for the maintenance of existing strains. The procedure is as follows:

- prepare 0.9% agar medium
- streak the algal sample onto the agar surface
- Incubate for 5 - 21 days
- select the best colonies and transfer them into a test tube
- incubation on an illuminated glass rack when a colour change is observed in the tube, check the isolated algal strain under microscope

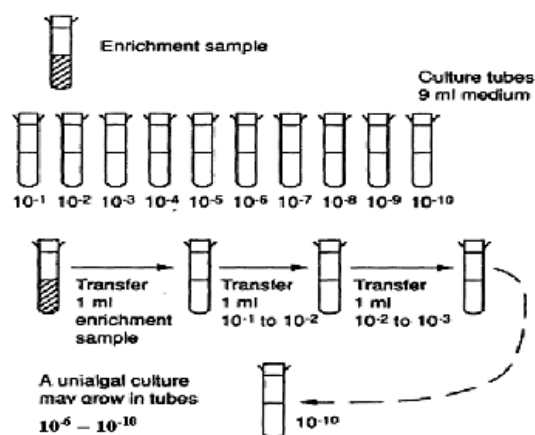


Agar plates with algal streaks

Serial dilution

Using aseptic technique, dispense 9 ml of media into each of ten test tubes with sterile automatic dispenser or sterile 10 ml pipettes. Label tubes 10⁻¹ to 10⁻¹⁰ indicating dilution factor.

- Add 1 ml of enrichment sample to the first tube (10⁻¹) and mix gently.
- Take 1 ml of this dilution and add to the next tube (10⁻²), mix gently.
- Repeat this procedure for the remaining tubes (10⁻³ to 10⁻¹⁰).
- Incubate test-tubes under controlled temperature and light conditions:
- Examine cultures microscopically after 2-4 weeks by withdrawing a small sample from each tube. A unialgal culture may grow in one of the higher dilution tubes e.g. 10⁻⁶ to 10⁻¹⁰. If tubes contain two or three different species then micromanipulation can be used to obtain unialgal cultures



Algal production

Physical and chemical conditions

The important parameters regulating algal growth are nutrient quantity and quality, light intensity, pH, turbulence, salinity and temperature. The optimal parameters as well as the tolerated ranges are species specific and a broad generalization is given in Table 1. Also, the various factors may be interdependent and a parameter that is optimal for one set of conditions is not necessarily optimal for another.

Table 1 A generalized set of conditions for culturing micro-algae (modified Anonymous, 1991)

Parameters	Range	Optima
Temperature (°C)	16-27	18-24
Salinity (g.l-1)	12-40	20-24
Light intensity (lux) (depends on volume and density)	1,000-10,000	2,500- 5,000
Photoperiod (light:dark, hours)		6:8 (min) 24:0 (max)
pH	7-9	8.2-8.7

Maintenance of stock and starter cultures

Stock cultures, otherwise known as master cultures, of the preferred species are the basic foundation of culture. They are normally supplied as monospecific cultures from reputed culture collections. Stock cultures are used as inocula when required. Every effort should be made to minimize the risk of contaminating the stock and starter cultures with competing microorganisms. The sterile procedures described below should be followed to ensure that contamination does not occur. Stock cultures are kept in small, transparent, autoclavable containers. For example, 500 ml borosilicate glass, flat-bottomed boiling or conical flasks fitted with a cotton wool plug at the neck, suitable for containing 250 ml of sterile, autoclaved medium, are ideal. The composition and preparation of Guillard's F/2 medium is given in Table 2

Table 2: Guillard's F/2 media used for culturing algae in bivalve hatcheries from Guillard (1975)

Nutrient	wt (gl-1)
Nitrate NaNO ₃	75.0
Phosphate NaH ₂ PO ₄ . H ₂ O	5.0
Silicate Na ₂ SiO ₃ .9H ₂ O	30.0
FeCl ₃ .6H ₂ O	3.5
Na ₂ EDTA	4.36
Dissolve in 900 ml distilled	H ₂ O

Add 1 ml of each of the following trace metal solutions

Trace metal	wt (g 100 ml-1)
CuSO ₄ .5 H ₂ O	0.98
ZnSO ₄ .7 H ₂ O	2.20
CoCl ₂ .6 H ₂ O	1.00
MnCl ₂ .4 H ₂ O	18.00
Na ₂ MoO ₄ .2 H ₂ O	0.63

Make up the volume to 1 l with distilled H₂O (pH ca. 2.0)

Add 1 ml per litre FSW of the above solutions (#1-4).

Vitamins	wt (mg l-1)
Biotin	1.0 mg
B-12	1.0 mg
Thiamine HCl	20.0 mg

Dissolve in 1 l distilled H₂O. Store under refrigeration

Add 1/2 ml of vitamin solution for every 1 l of FSW.

Stock solutions and salts

The culture media are referred to "working stocks" and "primary stocks". Working stocks are those whose aliquots contribute directly to making the final media. Primary stocks are normally made where several single substance solutions are then combined to form the working stock, eg. CuSO₄.5H₂O and ZnSO₄.7H₂O are two of the primary stocks used to make up the Trace Metal working stock in F/2 medium. It is suggested that all stock or starter cultures be grown with AR grade chemicals it is understandable that in mass culture applications (> 20 - 50 L), particularly for aquaculture, these chemicals may be too expensive when bought in bulk quantities. Stock solutions are made up by accurately weighing the prescribed amount of nutrient and dissolving in a specified volume of distilled water, if possible in a volumetric flask. Some nutrients will readily dissolve; others need heat and stirring to fully dissolve. In contrast vitamin stocks are heat sensitive and should not be subjected to heat treatment and should also be kept in the dark. Failure to fully dissolve the primary stocks of some nutrients such

as EDTA can lead to gross precipitation when these stocks are combined to make the media.

Nutrients come with different salts and hydration. For example, while copper and zinc may be two desired active constituents they are readily obtained from suppliers with either SO₄ or Cl₂ salts (i.e. CuSO₄ or CuCl₂ and ZnSO₄ or ZnCl₂). Some nutrients also come with different hydrations, i.e. the .nH₂O suffix. Substituting one form for another may have no effect on the growth of some microalgae species, but it can lead to poor growth in others and also lead to unwanted and time consuming precipitation problems as the overall ratio of salts in the medium has changed. Therefore deviating from the prescribed recipes is to be avoided and ordering the correct form is recommended.

Procedure for transferring algal cultures from flask to flask

- Wipe all inner surfaces of inoculating booth with 85% ethanol.
- Place all flasks that will be required in the booth; i.e. all flasks to be transferred from (the transfer flask) and flasks containing sterilized media to be transferred into (new flasks).
- Close booth and switch on ultra-violet lamp. Leave for at least 20 minutes. (It is not safe to look directly at ultraviolet light, so a dark cover should be placed over the plexi-glass (transparent acrylic plastic) viewing plate when the light is on.)
- Switch off lamp. Ignite small burner.
- Remove foil caps from one transfer and one new flask. Flame the neck of each flask by slowly rotating the neck through the flame.
- Tilt the neck of the transfer flask toward the new flask. In one motion, remove both stoppers and pour an inoculum into the new flask. Transfer approximately 50 ml for diatom species and 100 ml for flagellates. Avoid touching the necks of the two flasks. Never touch the portion of the stopper that is inserted into the flask. Once the inoculum is added, replace the stopper in the transfer flask. Slowly flame the neck of the new flask before replacing its stopper.
- Replace foil cap over the neck of the new flask. Using a waterproof marker pen, label the new flask with the algal species inoculated and the date of transfer.
- Repeat procedure for all flasks within the booth. Once completed, turn off burner and open booth.
- Remove all new flasks and place in the algal incubator or a well-lit area in the algae culture facility.
- The remaining inoculum in the transfer flasks can be used to inoculate larger cultures such as 4 l flasks or carboys. (from: Bourne, Hodgson and Whyte, 1989)

Starter culture management

Procedures for the maintenance of starter cultures (inocula) are almost identical to those described above. These cultures are specifically grown to provide inocula to start larger volume cultures needed to produce food. A line of starter cultures is originally set-up from the stock culture of the required species. Starter cultures, like the stocks, can be grown in 500 ml boiling flasks in 250 ml of culture medium. Because they are needed to provide inocula it is necessary to grow them quickly. They are grown at 18 to 23 °C with an illumination of 4 750 to 5 250 lux. Starter cultures are generally aerated with an air/carbon dioxide (CO₂) mixture.

Starter cultures are grown for variable periods of time prior to use. In the case of diatom species, which have short generation times, this period is from 3 to 5 days. For the majority of flagellates it is 7 to 14 days. When ready for use a starter culture is sub-cultured using sterile techniques, as previously described. Twenty to 50 ml, (depending on species and the density of the culture), is transferred to a fresh 250 ml culture – to maintain the starter culture line. The remainder is used as an inoculum for larger cultures (up to 25 l in volume) to be grown for feeding or as an intermediate step in the process of large-scale culture, where they in turn act as the inocula for much larger cultures. Larger volume starter cultures may be needed to inoculate large-volume production cultures. For clarity, cultures of between 2 and 25 l volume will be referred to as intermediate-scale cultures. As an example, a 200 l production culture will initially begin with a 250 ml starter of the required species which is then transferred when it has grown to a larger volume 2 to 4 l starter.

When a 200 l culture is about to be started, 200 to 400 ml of the 2 to 4 l starter culture is used to start a new 2 or 4 l starter culture and the remainder to start the 200 l production culture.

With larger volume starters it is advantageous to increase the level of illumination and to aerate the culture with an air/carbon dioxide mixture. It is advisable to dilute the medium to grow diatom species to a salinity of 20 to 25 PSU (practical salinity units, equivalent to parts per thousand) to obtain the best possible growth rates. Most flagellate species are best grown at about 30 PSU.

Intermediate-scale culture

Most laboratories and hatcheries requiring small volumes of algae for food use spherical glass flasks, plastic buckets or glass or clear plastic carboys of up to 25 l volume. These are generally operated as batch culture systems or semi-continuously. Batch culture involves the inoculation of the culture medium with the required species. The culture is then grown rapidly until a further increase in cell density is inhibited by the failure of the light to adequately penetrate the culture. The culture is then completely harvested, the container washed and sterilized and started again with a new culture. The semi-continuous method involves starting the cultures in the same way but instead of completely harvesting them when they have grown; they are partially harvested before the light limiting stage is reached. The harvested volume is then replaced with freshly prepared culture medium and the process repeated 2 or 3 days later. In this way the life of a culture is extended. With some of the hardier species, e.g. *Tetraselmis suecica*, cultures will last for 3 months or more with harvests of 25 to 50% of the culture volume 3 times each week. Batch culture is generally used for delicate species and the rapidly growing diatoms. Semi-continuous culture is mainly used with hardier species of flagellates.

Growth phases of cultures

Harvesting takes place in semi-continuous culture during the exponential phase of growth. Batch harvests are made generally at the peak of exponential growth as the cultures enter the stationary phase. An illustration of the meaning of these terms is given in Figure 19. In this case the species cultured is the large, green flagellate, *Tetraselmis*. At inoculation from the starter culture,

the starting cell density in the culture is 25 to 50 cells per ml (cells per microlitre). After inoculation these cells grow and divide increasingly rapidly as they acclimatize to the culture conditions. This acclimatization period, which lasts for 2 to 3 days, is called the lag phase. Once adapted to the conditions, the rate of cell division accelerates and increase in the number of cells in the culture is logarithmic. This period lasts for 4 to 6 days and is called the exponential growth phase. Cell division rate then slows as light penetration through the culture and/or nutrients become limiting. The culture then enters the stationary phase, which can last for many days in the case of flagellates or only for a short time for diatoms. Cultures of flagellates remain in this phase by the recycling of nutrients from dead and decaying cells, but in the case of diatoms, which may produce self-inhibiting metabolites, which attract bacterial growth, the culture collapses.

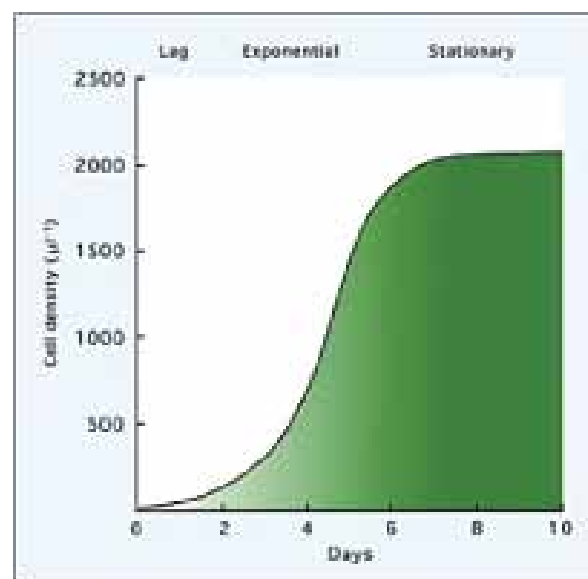


Figure: Phases in the growth of algal cultures illustrated by a typical growth curve for the large, green flagellate, *Tetraselmis suecica*.

Details of intermediate-scale culture operation

The complexity of the culture operation depends on the requirement for algae and the cost constraints within which the system needs to operate. In the simplest form the culture system may be just a scaled-up version of the starter cultures, using 2 l to 25 l flat-bottomed, glass flasks or carboys. These are part filled with the culture medium – in this case sterile, nutrient-enriched

seawater – and then they are inoculated with the required species and aerated with a mixture of 2% CO₂ carried in compressed air. The carbon dioxide is from a bottled gas source with gas pressure and flow regulation. This is to provide the carbon source for photosynthesis and to control pH within the range 7.5 to 8.2. The air/CO₂ mixture is filtered through a 0.2 µm porosity cartridge or membrane filter to remove the majority of air-borne contaminants and competing micro-organisms. The culture medium is prepared from filtered or sterilized seawater.

There are various options for culture water treatment:

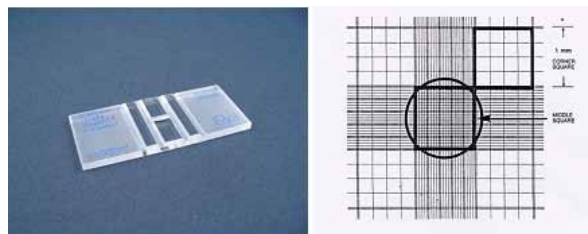
- Either the seawater is filtered to remove bacteria using 0.22 or 0.45 µm membrane cartridge filters, or,
- It is batch or continuously pasteurized at 65 to 75°C or,
- It is autoclaved at 1.06 kg per cm² for 20 minutes (After autoclaving the medium must be allowed to stand for 2 days in a suitable container closed from the atmosphere). Or,
- It is chemically sterilized with sodium hypochlorite solution at 25 mg per l free-chlorine (by adding 0.5 ml of domestic bleach – 5% sodium hypochlorite – per l of filtered seawater).
- Before use, the residual free-chlorine is neutralized by adding an excess of sodium thiosulphate solution (50.0 mg per l) prepared in distilled water.

Note: Methods (a) and (c) are most commonly used for small-scale culture preparation; (b) and (d), after prior filtration to 1 or 2 µm particle size, for large-scale culture.

After the sterilizing treatment, nutrient additions are made. Note that diatoms require the addition of silica (Si) to the basic nutrients. The medium is then ready to dispense aseptically to the culture flasks, which are then ready to be inoculated. To obtain the maximum productivity of most species it may be necessary to dilute the seawater with pure (distilled) freshwater (or from an uncontaminated source) before filtration or autoclaving. Growth and cell division rates of *Chaetoceros calcitrans*, *Thalassiosira pseudonana* and *Skeletonema costatum* are optimal at a salinity of about 20 to 25 PSU. Productivity of many of the flagellates is optimal at 25 to 30 PSU.

Estimating algal density

Accurate estimates of cell density can be made using a haemocytometer.



Haemocytometers are thick glass slides with two chambers on the upper surface, each measuring 1.0 x 1.0 mm. A special cover slip is placed over these two chambers giving a depth of 0.1 mm making the total volume of each chamber 0.1 mm³. The base of each chamber is marked with a grid to aid in counting cells within the area. Prior to counting motile algal species, 1 or 2 drops of 4% formalin should be added to a 10 to 20 ml sample of the culture to be counted. With the cover slip in position, one or two drops of the algal sample are introduced by means of a Pasteur pipette to fill both chambers. Cell density is estimated as follows. The central grid of each chamber (outlined in the circle) is sub-divided into 25 squares, each measuring 0.2 x 0.2 mm. The numbers of cells in 10 randomly chosen 0.2 x 0.2 mm squares are counted and the average or mean is calculated. This gives the mean number of algal cells per 0.2mm x 0.2mm x 0.1mm, or 0.004 mm³.

Example:

- Counts of algal cells: 40 + 30 + 50 + 60 + 55 + 65 + 70 + 45 + 40 + 70 = 525

Average = 52.5 cells per 0.004 mm³

- Multiply the average by 250 to give the average number of cells per mm³.
- Since there are 1000 mm³ in 1 ml, multiply the value calculated in B by 1 000.

In this example, the cell density would be 52.5x250 x1000 = 13.1 m (13.1 x 10⁶) cells per ml.

Extensive outdoor culture

Commercial hatcheries need to produce large volumes of good quality, high-food-value algae daily to support economic-scale seed production. Outdoor tank culture makes use of natural light.

Culture in rectangular or circular tanks with overhead illumination is used in shrimp hatcheries in India. This involves the fertilization of a large volume of seawater with the basic nutrients necessary for production, namely nitrogen, phosphorus and silica in one form or another. It is possible to induce monospecific blooms by prior fine ($<2\ \mu$ particle retention) filtration of the impounded seawater and the introduction of an inoculum of the required species, as long as it is hardy and vigorous. However, it is difficult to maintain such blooms for long periods because they rapidly become contaminated with other microorganisms.

Principles of large-scale culture management

The objective in culture management is to obtain the greatest possible daily yield of algae so that the culture systems are operated cost effectively. This yield must be sustained for long periods of time to maintain the hatchery output of post larvae. Ineffective management of algal culture greatly influences the potential for production and ultimately the selling price of the seed

Troubleshooting

Cultures will fail to grow, will become overly contaminated with competing micro-organisms or will crash even in the best-run hatcheries. Below are some pointers to check to determine the source of such failures.

- Air supply: Is there adequate air entering the cultures? Are the cells sedimenting to the bot-

tom of the culture vessel? This may happen when culturing certain diatoms, in which case the air flow rate should be increased. It should not happen in the case of commonly cultured flagellates. If it does, then the problem lies elsewhere.

- Temperature: Check min/max thermometer. Were there any increases or decreases in the temperature of the algal culture facility over the past 24 hours? Most of the commonly cultured algal species cannot tolerate temperatures above 26°C for extended periods – or temperatures below 12°C. Temperatures in the range 18 to 23°C are ideal for indoor.
- PH: Check CO₂ supply; Is the CO₂ cylinder empty? Check pH of the algal cultures using a pH probe. Is the pH too high (above 8.5)? Is the pH too low (below 7.5)? Adjust the CO₂ supply accordingly.
- Nutrients: Check records for the last time the cultures received nutrients. This is particularly important for semi-continuous cultures.
- Contamination. Are the walls of the culture container, particularly at the water/air interface, visibly foaming or fouled with what appears to be detritus? If so, the culture is at the end of its useful life and needs to be replaced. If this is a continuing problem in the early stages of the culture cycle with a particular species, then check the starter cultures for signs of contaminating organisms and replace them as necessary.

Zooplankton for marine fish larval feed

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Introduction

Larval nutrition is an important part of the hatchery operation. Successful larval rearing primarily depends on the live feed and zooplankton forms the most important component in the live feed. Larval feed should be smaller, easily digestible, rich in nutrients and allow autolysis. Formulated feed may not meet all these requirements and in most of the cases this will reduce the larval survival rate than live food. Moreover, the fish larvae have poor vision and less developed olfactory and digestive organs. The size of feed is important when the mouth size of larvae is concerned. Poor vision, improper digestive system and weaker movements make it difficult for the larvae to get proper nourishment. Some fish larvae (precautionary type) are with good yolk reserve and they start feeding at a comparatively developed stage while some others (atresial type) start feeding at smaller size and lesser developed stage. Salmon, cobia and clown fish larvae are comparatively larger than grouper and damsel larvae. Live feed in continuous movement in the water will help the weaker larvae to prey upon these tiny organisms. Copepods, cladocerans, decapod larvae, rotifers and ciliates are the important zooplankton organisms which form the food of fish larvae in the wild. The most popular zooplankters used for fish larvae are rotifers, artemia and copepods.

Rotifers

Rotifers are larval live feeds used in both marine and freshwater hatcheries. Rotifers are very small organisms mostly ranging from 0.1 to 0.5 mm and belong to the Phylum Rotifera. *Brachionus plicatilis* and *B. rotundiformis* are the common species used in hatcheries all over the world. Rotifers are filter feeding organisms with high reproduction rate, capable of both sexual and asexual reproduction and can be reared in large densities upto 2000 animals/ml. *B. plicatilis* and *B. rotundiformis* have three strains developed for hatchery purpose; i) L type with lorica ranging from 100-340 μ ; ii) S type with a size range of 100-210 μ and iii) SS type with less than 100 μ size.

Ideal water quality parameters for maintaining successful mass production of *B. plicatilis* are -salinity below 35 ppt, temperature 20- 28°C, dissolved oxygen above 3 mg/l, pH above 7.5 and ammonia below 1 mg/l. Major contaminants in the culture are ciliates and bacteria. Bacterial load especially of *Vibrio* sp. should be below 107 CFU/ml. The culture should be free from ciliates like *Uronema* sp and *Euplotes* sp. In case of severe contamination, washing through a flow through system with 50 μ mesh plankton net can regain the pure culture. Intensive indoor culture is mainly by batch-culture using microalgae as feed.

Mass cultures of rotifers always carry some risks of sudden mortality. Hence it is ideal to maintain stock culture separately under aseptic conditions. Rotifers for starter culture can be collected from the wild and isolated through a series of antibiotic treatments and purified culture without any contamination can be prepared. It is always easy to start the culture by taking a small sample from a well maintained stock culture in a hatchery or from a laboratory. All the culture tubes and filters should be properly sterilised before going for stock culture. The stock should be maintained at 28°C with proper illumination of approximately 3000 lux using *Chlorella* as feed. It is ideal to add fresh algal culture on daily basis to these tubes. It is better if all the culture tubes are placed on a gentle shaker or a rotating shaft for providing sufficient oxygen. Ideally this should be maintained at a density of 2 rotifers/ml upto 200 nos/ml. The stock culture should be periodically re-cultured and disinfected using mild antibiotic as and when it requires. Once the density reaches around 200 nos/ml, this can be transferred to Erlenmeyer's flasks of 500 ml capacity with an algal concentration of 1.6×10^6 cells/ml. Approximately 50 ml of the algae should be added daily and no aeration is required during this short rearing period. The concentration will reach 200-300 cells/ ml within 3 days period and now the culture is ready for inoculation to 15 l bottles. The culture should be passed through first strainer of 200 μ mesh and then strained using 50 μ mesh and the filtrate can

be transferred to 15 l bottles with 2 l water and a density of approximately 50 nos/ml for producing starter culture. This stage onwards we should go for aeration. Fresh algae of concentration of 1.6×10^6 cells/ml should be supplied as daily ration. Within 7 days the 15 l bottle will be full and the culture is now ready for mass culture.

The culture can be maintained using fresh algal culture/ commercial algal pastes/ or with baker's yeast/ formulated diets. Ideal formulated diets for rotifers are now available in market and Selco is one such company producing rotifer feeds. For mass production of rotifers, the hatchery should have facility for providing at least any one of the above feeds. Mass culture is generally maintained in large indoor tanks. Continuous harvest is possible if the rotifer reaches a density of 300-500 nos/ml. The rotifers will double its population daily. Different types of sieves/ strainers prepared using 50μ mesh net can be used for filtering the mass culture during harvest. Algal culture should be pumped in the culture tank on a daily basis and enough aeration should be given to maintain the production.

Nutritional value of rotifers mainly depends on the type of feed used. Rotifer cultured using Tetraselmis, Nannochloropsis or Isochrysis or a mixture of these will be higher in DHA and PUFA content than that cultured using Chlorella. Several commercial products are also available for enrichment of rotifers. Use of enriched rotifer for feeding larvae is essential for better larval survival. Harvested rotifers can be reared separately in water containing enrichment media. Simple enrichment can be done using Tetraselmis, Nanno-



Lorica of *B. plicatilis*

chloropsis or Isochrysis or a mixture of these fed finally for one day to the rotifers. Commercially available enrichment media can be added to the harvested rotifers kept in higher concentrations with minimum water for few hours. The enrichment status can be observed by the colour change of the rotifers used or by biochemical estimation of PUFA levels using a gas chromatograph. The enriched rotifers can be directly fed to fish larvae.

Artemia

Artemia or the brine shrimp which has the ability to make dormant eggs called cysts is the world's most popular and widely used live feed. The artemia cysts can be stored in dry condition for a longer period and over 200 tonnes of artemia cyst is marketed annually all over the world. Artemia is typically a primitive crustacean belonging to the class Branchiopoda with a total length of about 0.7-1.2 mm and sexes separate. Artemia are produced in hyper saline ponds and can tolerate wide range of salinity and temperature but with an optimum requirement of 35-38 ppt. Artemia can reproduce parthenogenetically and in adverse conditions, it produce dormant cysts (chorion coated) which can be stored in dry condition without losing its viability for more than 2 years.

In dormant condition artemia cyst is round but concave at one or two sides. On hydration this will become spherical and in less than 24 h hydration and aeration, this hatches out into nauplii. Freshly hatched nauplii is Instar I with a length of $400-500\mu$ and is popularly used for feeding the larvae. Within 7-8 h this will change to Instar II and start feeding on minute algae. The larvae



Brachionus plicatilis (live)

again undergo 13 more moults to become adult artemia. Artemia is a filter feeder mainly feeding on microalgae.

Each gram contains 200000 to 300000 of artemia cysts and almost 50% will hatch within 20-24 h on proper hydration. The artemia cysts must be properly weighed and kept for hydration in normal seawater of salinity not less than 35 ppt. The density can be 2 g/l and the pH should be above 8 and the temperature around 28°C. Strong aeration and illumination (above 2000 lux which can be achieved using fluorescent tubes) are essential for ensuring maximum hatching. Depending on the volume of larval rearing tank and the species under culture, requirement of artemia nauplii should be calculated. Daily measure the artemia nauplii left over in the tank by examining water in the larval rearing tank and back calculate the requirement of nauplii/l and requirement of cysts in g for producing that amount of nauplii.

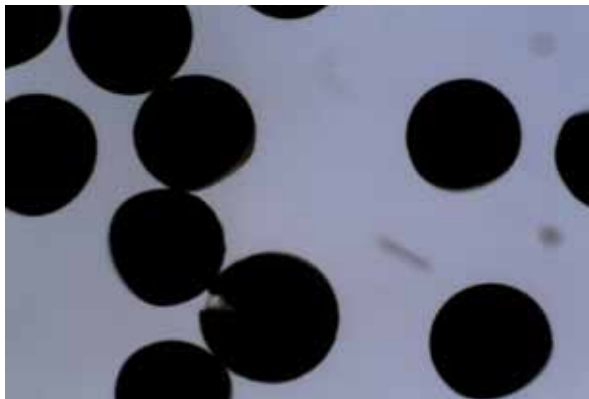
Artemia nauplii if required in large quantities, it is essential to decapsulate the cysts before hydra-



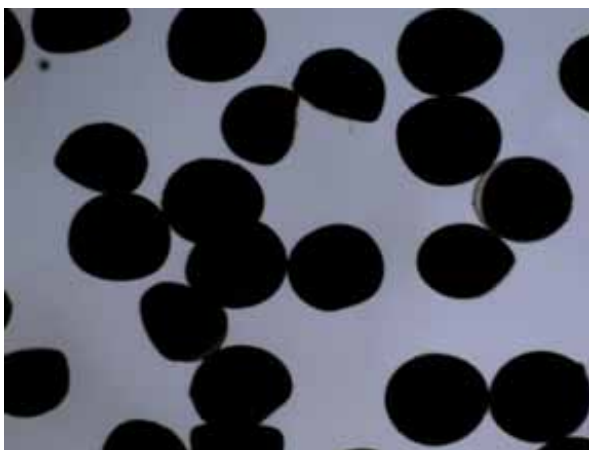
Artemia Instar I



Artemia Instar II



Artemia cysts before hydration



Artemia cyst after hydration

tion to increase the quality and quantity of hatching. Soak the cyst in 200 ppm sodium hypochlorite solution for 30 minutes and wash thoroughly with tap water using 125 μ sieve. Cylindroconical tanks are ideal for hatching and aeration should be from the conical tip of the tank. Remove aeration prior to harvesting of nauplii and since they are phototactic, can be easily aggregated using light.

Artemia cysts can be decapsulated and directly used for feeding the fish larvae or this can be stored at 4°C for 1-2 weeks without losing its viability. Decapsulation process is simple but need constant observation. Sodium hypochlorite solution (0.5g/l) or liquid bleach (5ml/l) are commonly used for decapsulation. Decapsulation process should be monitored properly. Keeping longer duration in bleaching agents will affect the survival. The entire container should be immersed in ice cold water so that temperature inside the container should be below 20°C. The time required for decapsulation process will vary from 5 to 15

minutes. The cysts will turn grey with powder bleach and orange colour with liquid bleach, few samples should be observed using a stereo microscope and if the cyst wall is dissolved, the cysts should be rinsed using 125 μ sieve several times in water till there is no trace of chlorine. In order to ensure the removal of chlorine, wash the cysts in 0.1N HCl or 0.1% Sodium thiosulphate solution (Na₂S₂O₃) for one minute. Finally wash through clean filtered seawater and check the water using chlorine test kits and chlorine free cysts can be directly fed to the fish larvae or can be kept for hatching or this can be sieved and stored in refrigerator at 4°C.

Artemia nauplii are nutritionally poor when compared to copepod nauplii and this can be enriched using PUFA and DHA by the same method of enrichment of rotifers. Artemia biomass can be regularly produced using microalgae in tanks with natural sea water. This can be fed by algal paste or fresh algae. All stages of artemia can be cultivated in large scale and can be harvested regularly using normal seawater without much effort.

Copepods

One of the major problems in culture of marine fishes is lacunas in development of complete larval feed. In hatcheries, rotifers and brine shrimps are used as live feed for marine fin fishes during the early life stages. Apart from these organisms, due to the presence of desirable characters such as size and nutritional value, copepods also play a role in larval rearing. Copepods are tiny planktonic crustaceans with more than 10000 species living in a variety of ecological niches. They are a good source of proteins, amino acids, fatty acids, vitamins and minerals and the nauplii are successfully used as first feed for fish larvae in cases where rotifers were inadequate in their nutritional value. Copepods contain the essential unsaturated fatty acids (HUFA) which makes them more appropriate food for fish larvae. Most of the early fish larvae are evolutionarily adapted for feeding on copepods than on other animals.

Copepods are successfully cultured in finfish hatcheries of many countries especially for feeding atresial larvae of certain fishes like groupers. Cultured species includes calanoid as well as harpacticoid copepods. In a study conducted on the wild-caught fish larvae, it was revealed that calanoid copepod nauplii were an essential item

in the early feed of many fish species. Calanoids of the genera *Acartia* and *Gladioferens* have been proved as important live feed for improving survival of some fish species. Promising species are found in the genera *Centropages*, *Acartia*, *Labidocera* and *Eurytemora*. On the other hand, Harpacticoid copepods also are a good source of larval and juvenile fish feed in aquaculture. Alone or as a supplement, in many cases harpacticoids have been proved to improve primary growth than rotifers and brine shrimps. The morphological minuteness of harpacticoids enables their feeding by gap-limited fish larvae such as the groupers and snappers. The presence of rich natural fatty acids in it increases the value of copepod in aquaculture. At commercial scale, only a few copepods have been successively reared. Due to several reasons, most of the copepod rearing trials is done in small scale lasting for few weeks or months only. Modern technologies by means of pond or bag culture ; with an input of large quantities of sea water or by the placement of bags of various mesh sizes in open sea, copepod culture can be successfully enhanced.

Due to their compatible size and suitability to culture technologies, harpacticoids seems to be the most suitable one for culture. In natural environment, harpacticoids exhibit a detritivorous behavior and they are adaptable to both formulated artificial feeds and algal cultures and can be grown upto a density of 1,15,000 individuals L⁻¹. Calanoid species such as *Acartia tonsa*, *Calanus hamatus*, *Eurytemora affinis* and *Gladioferens imparipes* have already being used as larval fish feed. Along with these, symbiotic copepods also have a role in the marine finfish rearing. Copepods of the family Mycolidae like *Pseudomyicola spinosus* indicates that, this species has a potential for development into a live feed for finfish larvae due to its easy availability, planktonic naupliar larvae, suitability to culture and its property of having maximum fecundity rate. Thus, the naupliar larvae of *P. spinosus* is largely cultivated and released in a mussel bed to improve its population. Considering their importance mass culture of several copepods are being taken up which are beneficial for aquaculture.

Species popularly cultured

Of the planktonic copepods in estuarine and coastal habitats, calanoids are the most abundant

taxa of pelagic realm forming an extreme connecting link between phytoplankton and fishes in the inshore ecosystem. Among calanoids, the easier one to cultivate is the *Acartia* spp. than *Calanus* spp. and *Temora* spp. Most of the species present in Calanoida are of ≈ 1.0 mm total length, with a size range of 0.4-10.0 mm. *Acartia clausi* and *Calanus finmarchicus* are the most widely studied calanoids followed by *Temora longicornis*, *Paracalanus parvis*, *Calanus helgolandicus*, *Pseudocalanus elongates*, *Acartia tonsa*, *Centropages hamatus*, *Centropages typicus* and *Temora stylifera*.

Based on studies conducted by the Japanese planktologists and aquaculture scientists on the improvement of copepodal massculture, 13 species were recommended for mass cultivation. These includes *Acartia clausi* (*A. hudsonica* or *A. omorii*), *A. longiremis*, *Eurytemora pacifica*, *Euterpina acutifrons*, *Microsetella norvegica*, *Oithona brevicornis* (*O. davisae*), *O. nana*, *O. similis*, *Pseudodiaptomus inopinus*, *P. marinus* and *Tigriopus japonicas*. Among these, *T. japonicas* is the only one which is produced on a large scale and used in marine fish farming. Maintenance of cultivation has a lot of difficulties including frequent replacement of water, high demand for algal diet as well as low and unstable population growth.



Pseudodiaptomus serricaudatus

Culture methods- Harpacticoids & Calanoids

Copepod culture can be done by continuous as well as batch culture. Normally coastal species with shorter life span and with a wider tolerance to salinity and temperature alterations are preferred for aquaculture. In the late 90's, Taiwan

was the first country to start copepod culture. Without water exchange, copepods can be grown in ponds with low salinity. Some culturists are of the opinion that the ponds with clay are more suitable for these planktonic blooms as the beneficial nutrients are available. Even sandy ponds are found suitable as culture field. In routine culture, rotifers can be cultured to a high density of 2000 nos/ml. Many copepods can be cultured commercially at a density of 20-90 nos/ml. These include *Paracalanoid* spp. *Bestolina simili*, *Parvocalanus crassirostris* and a harpacticoid, *Eupertina acutifrons*. The culture technique of each copepod is different.

More than 60 copepod species have been raised in laboratory. For promoting the culture and improving cost-effectiveness of marine copepods in aquaculture industry, the development of appropriate culture techniques is essential. Copepods can be cultured extensively, intensively and semi-intensively. Copepods can be extensively developed in tanks, outdoor ponds, lagoons or enclosed fjords. By using appropriate mesh sizes these cultured copepods can be made available to fish larvae. Planktonic copepods including *Acartia*, *Centropages* and *Temora* can be cultured in such systems. In extensive systems, culture is done normally on the basis of microalgal blooms induced by agricultural fertilizers. Occurrence of parasitic infections on most species is the major problem in extensive copepod production. Due to risk of parasitic transmission harvesting copepods from natural environments is not desirable. As copepods are also natural prey for fish in aquaculture ponds, they can be used semi-intensively on an industrial scale.

In intensive culture methods, we can get reliable and sustained production. Long generation-time of most species is the major problem in intensive production of copepods. For intensive mass production, Harpacticoids are recommended as the suitable ones than Calanoids. Harpacticoids are promising species for intensive cultivation due to their tolerance to temperature and salinity, ability to feed on a large amount of live and inert diets and high fecundity rate with relatively short life cycle (5-29 days). They can also be cultured in high densities (exceeding 100,000 individuals per litre) as they possess planktonic nupliar stages and have capability to utilize wastes. But calanoid culture is more beneficial than harpacticoids because of their pelagic

behavior and as they are the most readily available prey to fish larvae. By providing appropriate temperature, sufficient live feed (algae) as well as frequent exchange of seawater with the use of advanced mesh of varying measurements, continuous and a reliable supply of large scale copepods can be got.

Temora turbinata

Temora turbinata, a common calanoid copepod is an important food for many fish species. This species has been reported as an important candidate species for mass culture. *T. turbinata* has capacity to tolerate wide ranges of environmental conditions. This is a slow moving non-myelinated calanoid copepod species which shows very low escape reflex. Mass culture of *T. turbinata* has been standardised by CMFRI. The culture started with a few isolated specimens from the plankton sample in a beaker, then to buckets and now in sintex tanks of 1000 liter capacity. Such tanks are maintained in the hatchery. The culture is fed with a mixture of *Nannochloropsis* and *Isochrysis*. This species has several advantages over several other species tried here. It has no brood pouch or egg sacs. Eggs are scattered in the culture medium and can be collected easily by collecting the bottom samples. Cannibalism is very rare in all stages of their life. This species is very hardy and can even tolerate ciliate contamination up to certain extent.

T. turbinata takes almost 17-18 days to mature. It has six naupliar stages and 5 copepodite stages. It is difficult to distinguish the sexes. Males have slightly different antennules and caudal ramus, cephalothorax is slightly slender and the fifth leg of male is totally modified. Females are more common in collections.

Collection and Isolation

Collection was done using plankton net during early hours and the efficiency of collection procedure mainly depends on the mode, time and location of collection. The marine copepod *T. turbinata* was collected from plankton collected using 330 μ m mesh plankton net. At that time, the water temperature was around 24-26c and salinity range was 34-35 ppt. The generation was enhanced by continuous culture maintained in the hatchery for many months with a nutritional supply of *I. galbana* and *N. oculata*. From the planktons collected, copepods with similar features

were picked up by using a fine dropper under a stereo dissecting microscope. Different species were sorted out and maintained in monoculture for evaluating their adaptability for mass culture. The Calanoid copepod, *T. turbinata* has satisfied all the sufficient characters for mass culture; with a size ranging from 60 to 440 μ m, which can be used as a feed for many species of marine fish. This can be cultured up to 4-5 nos ml⁻¹ in a temperature range between 27 °C to 32 °C and a salinity of 30-32 ppt.



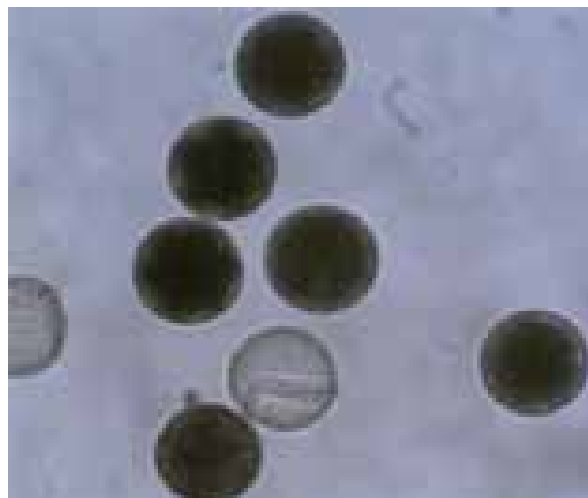
T. turbinata (male)



T. turbinata (female)

Culture

Culture was started in 500 ml beakers and later transferred to 1 liter beakers. Under different culture conditions copepods can be raised. Mass culture of *T. turbinata* was done in five 500 liter tanks. Tanks were filled using chlorine treated and de-chlorinated water filtered through a 5 micron filter bag from an outdoor tank of 200 m³. To the contamination-free water the resident population of *T. turbinata* has to be introduced. The tanks should be placed in 60% shade. Using a refractometer, the salinity of the resident water should always be maintained at 30 ppt (+/- 2 ppt). The optimum temperature should be maintained at 27 - 30°C. A mixture of *I. galbana* and *N. oculata* has to be supplied regularly. This helps in preventing contamination of the culture system by ciliates upto a certain extent. Mild aeration is also required in these tanks. Siphoning off of the sediment from the tanks on alternate days are required to prevent ciliate growth, thereby enhanc-



T. turbinata eggs

ing population. By passing through a series of filtering mesh of 20 μ , 110 μ and 330 μ animals with different ages, ovigerous females and mating pairs were sorted out and brought for further culture from the sediments collected.



Naupliar stages of T. turbinata

Feeding

Supply of an optimal diet is an important factor for culturing copepods. By means of gut analysis and faecal examination the feeding of copepod can be determined. While selecting food for a species the particle size as well as the digestibility of the feed has to be taken into consideration. The chemical composition of the algal feed may also be considered as it may also have some effects on survival. In the case of *T. turbinata* the particle size should not be more than 10 μ . The ideal algal

feed for *T. turbinata* was *I. galbana* and *N. oculata* (\approx 2-5 μ). For feeding, stock and mass cultures are prepared. Using a compound microscope, supply of unicellular algae can be quantified. The amount of food required is directly proportional to the copepod biomass present in the culture media. Feed of 2 x 10⁴ cells/ml/day is sufficient for young nauplii. Then, it can be gradually increased upto 1 x 10⁵ cells/ml/day till they mature. Daily assessment of population density is essential. If the water appears cloudy, the feed rate should

be decreased. The supply of sufficient amount of feed will successfully facilitate the peak production.

Cleaning

The major threat occurring to the copepod culture is ciliate infection. Total removal of ciliates is an impossible task. So, by means of proper cleaning, it can be avoided. Daily removal of accumulated fecal debris, wastes and uneaten food materials can be done by siphoning. The siphoned water has to be collected in separate buckets. The buckets should be contamination free. Later the supernatant portion of the filtrate should be filtered through a 100 micron filter to recover adults if any. The bottom filtrate can be stored in buckets with mild aeration for 3-4 days. Every day the developed copepod nauplii in the buckets were filtered using a 150 μ and 20 μ mesh, washed thoroughly in freshwater for 30 sec and also in seawater. The adults if any collected in 150 μ filter can be deposited into the main tank itself. The young nauplii collected through 20 μ filter can be washed with seawater and put in another fresh bucket for further growth or it can be used for feeding. Renewal of resident seawater should be done in every 2 weeks and a replacement of tank to be done in every 4 weeks for preventing ciliate development. Although, the volume to be removed is not critical, the exchange of 10% of water is most effective at the time of removing debris from the bottom.

Harvesting

It may take about one month for a tank to reach a density of minimum 1000 copepods/l and only then it becomes ready for a harvest. In a harvesting, there may be eggs, nauplii and adult copepods. In a continuous culture, 5-10% can be regularly harvested. The harvesting can be done by siphoning out the water. Production can be enhanced by proper cleaning and adequate supply of feed.

Problems

The main problem in *T. turbinata* culture is the ciliate infestation in tanks. Overfeeding, fecal contamination and accumulated debris results in emergence of ciliates. Ciliate growth can be assessed by the cloudy nature at the bottom of the tanks. The deficiency of feed is another problem in the decline of culture population. The feed provided has to be proportional to the biomass present. Adults feeding on eggs and early larvae will result in total collapse of the population if sufficient feed is not provided. The feed provided should be contamination free, especially of ciliates. If a mesh of 20 μ is used for filtering the feed, it will help in preventing ciliates to an extent. Matured algae should be fed to attain a successful growth. Immature or collapsed algal feed shall lead to a decline in population. The settled debris and accumulated wastes in resident sea water is also a substrate for development of ciliates and other dangerous organisms. So renewal of sea water in resident tanks is essential to create a healthy environment for the cultured species.

Hatchery protocols for seed production of cobia and pompano

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Introduction

Many Southeast Asian countries, European nations and Western countries like USA have developed commercial marine finfish hatchery technologies for many commercially important species namely groupers, salmon, tilapia, sea bass, sea bream, snappers, mullets, Chanos, etc. They have developed capacity to produce large and dependable quality of fish seeds which is the key for establishing reliable and sustainable marine finfish aquaculture sector. In India, technology for production of marine finfish seeds is in primitive stage except for sea bass. The Mandapam Regional Centre of the Central Marine Fisheries Research Institute (CMFRI) has developed hatchery technology for cobia, *Rachycentron canadum* during March 2010 for the first time in the country. Again the Centre has developed hatchery seed production technology for the silver pompano, *Trachinotus blochii* for the first time in the country. Both the technologies are standardised and hence the CMFRI has entered into agreements with interested entrepreneurs and farmers for dissemination of technologies for development of cobia and silver pompano aquaculture sector in our country.

The cost-effective hatchery technologies developed by the Mandapam Regional Centre of the CMFRI comprise

1. Induced spawning protocols
2. Appropriate live feeds for larval rearing,
3. Commercial-scale protocols for larval rearing,
4. Nursery and grow-out culture protocols

The larval rearing procedures of cobia and pompano are described below

Egg harvest

The fertilized eggs of cobia and pompano float and are scooped gently using 500 μm net. To minimise collection of poor-quality eggs, which usually float deeper in the water, it is advisable to collect only the eggs which float at the water surface. Therefore, aeration can be switched off allowing the unfertilized / dead eggs to settle at the bottom of the tank. The floating layer of eggs thicker than one cm should be avoided. A thicker layer may reduce oxygen supply to the eggs, leading to possible anoxia after a short time. The eggs should be sampled and examined for their quality, number and developmental stages. The embryonic development inside the eggs could be studied using a microscope.

Check for the following egg characteristics:

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

Incubation of eggs

Incubation of eggs is done in incubation tanks of 3-5 tonne capacity. After hatching, only the hatched fish larvae have to be moved to the larval rearing tanks filled with filtered seawater. Prior

to this, the aeration should be stopped briefly to enable the debris and exuviae to settle at the bottom which can be removed by siphoning. Aeration needs to be adjusted suitably, not too strong to avoid excessive physical collision among eggs, but should be sufficient only to keep the eggs suspended in water column. The main purpose of aeration is to prevent clumping and settling down of eggs. Air bubbles should not be too small, as seen while using air diffusers instead of stones, as it results in clumped eggs and damage of the eggs.

Stocking density can be maintained at a moderate level of 200 to 500 eggs per litre. The hatching of eggs takes place from 18 to 24 hours. As the fecundity is normally high in cobia, we may require more incubation tanks, whereas pompano requires only one tank/ female.

The embryonic developmental stages of cobia and pompano normally look alike except for the duration of development and size of the larvae. The photos of embryonic development and newly hatched larvae are provided below;

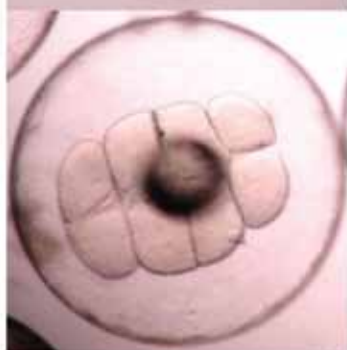
Two-cell stage



Four-cell stage



Eight-cell stage



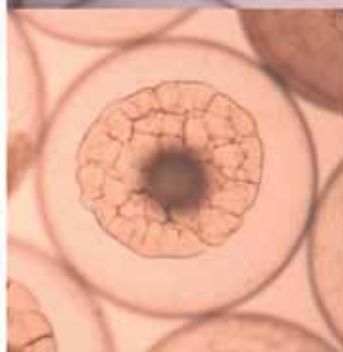
16-cell stage



32-cell stage



64-cell stage



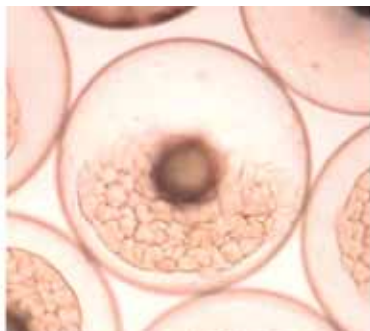
Early Morula



Late Morula



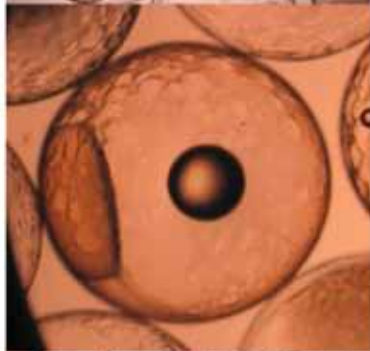
Early
Blastula



High



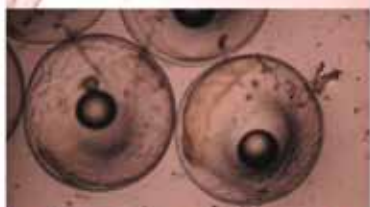
Dome



Oblong



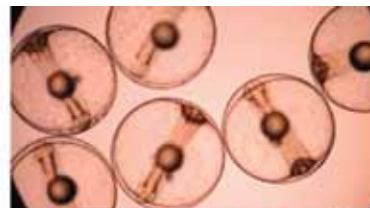
Epiboly



Early
Gastrula



Mid Gastrula



Late Gastrula



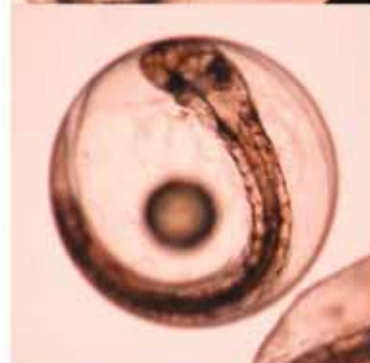
Bud



Segmentation



High-pec



Hatching in progress



Newly hatched larvae



Larvae-12 hour post hatch



2dph



Larviculture

The marine fish larvae are generally classified into altricial and precocial type. The altricial type of larvae is having very less yolk reserves at hatching and hence, the larvae are in an undeveloped stage when the yolk sac is completely resorbed. The development of digestive system is also very primitive in these types of larvae. Many of the marine fin fishes which are suitable for aquaculture are having the altricial type of larvae which poses challenges in their larviculture. When the yolk reserves are fully exhausted, the larval size and mouth gape are very small and the perceptive powers for searching and taking external feed is also very less. The period when the yolk reserves are fully exhausted and larvae need to resort to

exogenous feeding is a critical period in the larviculture of most marine fin fishes. Unless proper live feeds of required size is provided in sufficient densities in larviculture media and its nutritional requirements especially in terms of PUFA are met, large scale mortality is bound to happen at this stage and hence it is evident that the larviculture of marine finfish having altricial larvae is really challenging and proper management of live feed is the most vital pre-requisite for the success in terms of survival and growth of the larvae.

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

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

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

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

Larviculture of cobia

Newly hatched larvae of cobia normally measures 3.4 mm size. Larval mouth opens at 3-5 days post hatch (dph). Metamorphosis starts from 18-21 dph. Newly hatched cobia larvae generally start feeding at 3 dph and they can be fed with the enriched rotifer (*Brachionus rotundiformis*) at the rate of 10-12 nos / ml, two times a day till 10 dph. From 8 dph, the larvae can be fed with enriched *Artemia* nauplii at the rate of 2-3 nos / ml, 2 times a day. During the rotifer and *Artemia* feeding stage, green water technique can be used in the larviculture system with the microalgae *Nannochloropsis oculata* at the cell density of 1×10^7 cells / ml. The weaning to artificial larval diets has to be started from 15- 18 dph. While weaning, formulated feed should be given 30 minutes prior to feeding with live feed. Size of the artificial feed has to be smaller than the mouth size of the fish. Continuous water exchange is required during weaning stage. Between 25-40 dph, the larvae are highly cannibalistic and hence size-grading has to be undertaken at every three days interval. During this stage, the fry could be weaned totally to artificial diets. Larval rearing can be practised both intensively in tanks and extensively in ponds. The major factors affecting the growth and survival of larvae are nutrition, environmental conditions and handling stress. Since there is high demand for essential fatty acids (EFAs), enrichment protocols are needed for live-feeds. The water exchange can be practically nil till 7dph and it can be gradually increased from 10-100 % from 8 to 12 dph. But, tank bottom siphoning should be

carried out from day 1. The environmental conditions required during the larviculture period are DO₂: > 5mg / l, NH₃: < 0.1mg / l, pH: 7.8 – 8.4, Salinity: 25-35 ppt, water temperature : 27-33° C.

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

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

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

Nursery and grow-out rearing of cobia

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

Larvae and fingerlings are shown in the given plates (dph = day post hatch)



2dph



4 dph



6 dph



10 dph



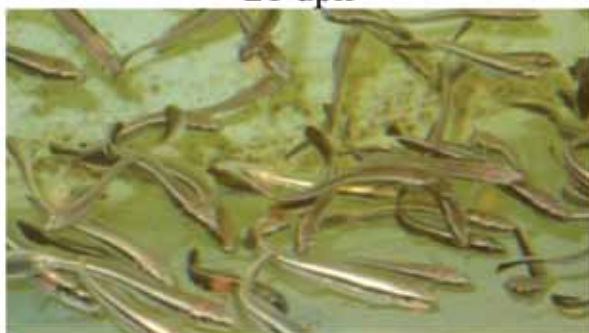
13 dph



15 dph



21 dph



45 dph



40 dph



62 ph

Larviculture of Pompano

The newly hatched larvae were stocked at a density of 15000 larvae in FRP tanks of 2 m³ capacity filled with 1.5 m³ filtered seawater. The tanks were provided with mild aeration and green water at a cell density of 1 x10⁷/ml. The mouth of the larvae opens on 3dph and the mouth size was around 230 μ .

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

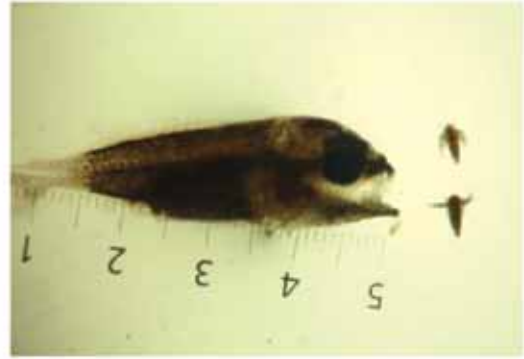
Nursery Rearing of Pompano

Nursery rearing could be initiated from 25 to 30 dph. At this stage, artificial feed of 800 μ size could be provided. Thereafter, fingerlings were fed with progressively higher size range of floating extruded larval feeds. Daily water exchange of 100% is advisable. Water quality parameters like salinity, temperature, pH, Oxygen level and ammonia are closely monitored during the entire larviculture period. After 55dph, the fingerlings with size range from 1 to 1.5 inch size can be supplied to farmers for stocking in the happas / tanks for further nursery rearing and grow-out farming thereafter. The pompano fingerlings can be reared at salinities as low as 5 ppt. At lower salinities i.e. from 10 to 15 ppt, they grow faster than in pure seawater.

Larval stages of pompano are shown in the plates below



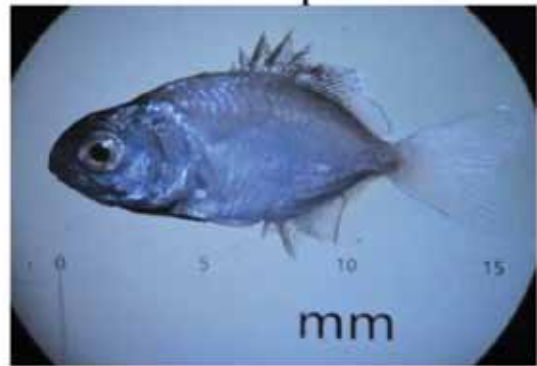
3 dph



10 dph



15 dph



25 dph

**Development of innovative low cost cages
for promoting open sea cage
culture along the Indian coast**

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Development of innovative low cost cages for promoting open sea cage culture along the Indian coast

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Introduction

Capture fisheries is undergoing tremendous changes either due to increased fishing pressure or due to decrease in the production of certain groups due to fishery dependent or fishery independent factors. In spite of increasing effort the catch of almost all commercially important fin fishes and shell fishes is on the decline and results in severe resource depletion and unemployment. Fishermen community solely depending on fishing for their livelihood is facing an uncertain future. Decline in marine capture fishery also affects the availability of cheap protein for the masses and

also affects the GDP growth of the country. Open Sea Cage Culture is one answer to address this problem partially. India has a cost line of 7517 kilometers where open sea cage culture can be initiated at selected places where these systems will not clash with the fishing operation of the traditional and mechanized sector. Since cage farming is done in open waters where wave action and current takes care of the day to day maintenance of the cage cultured fishes high stocking densities leading to very high production is possible in open sea cage farming. Unlike pond culture the carbon foot print in cage culture is relatively low and therefore more eco friendly.

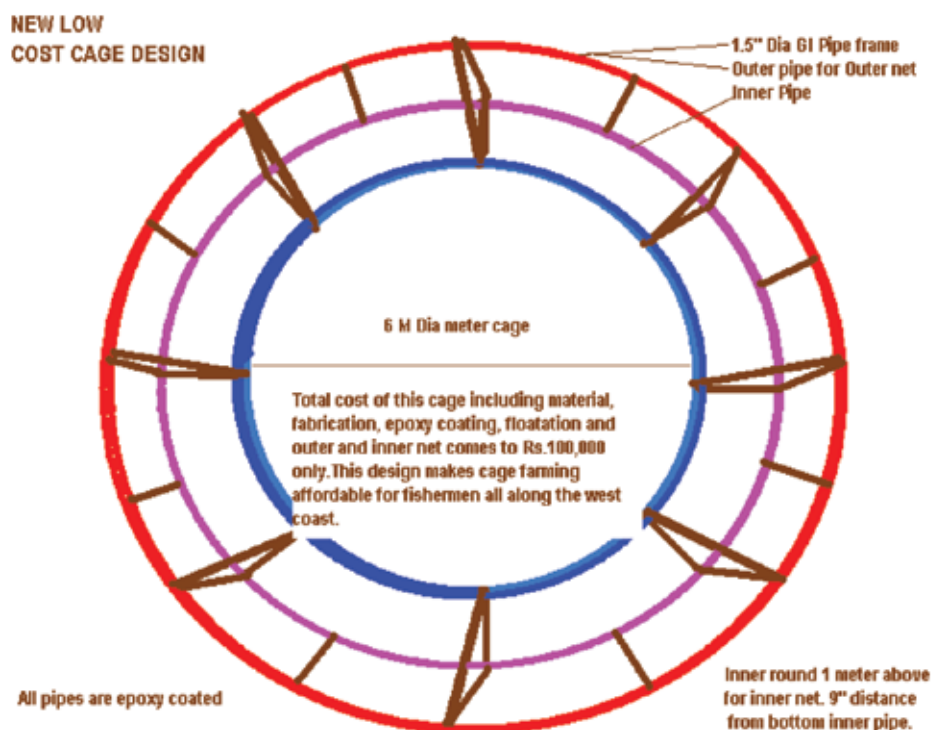


Fig.1. Design details of the low cost cage

In the western countries where cage farming technology is well advanced, the farming is mostly for Salmon. Salmon takes two years to reach a marketable size of 2 Kg. where as in the Indian conditions Sea bass reaches 2 Kg in 6 months and Cobia reaches 4 Kg in 6 Months. Hence the biomass that can be produced is almost four times in Indian conditions when compared with temperate waters and the cost of production is only one fifth when compared with western countries. The fishes that are being cultured in cages are high value fishes hence there is huge export potential also for cage cultured fishes.

Central Marine Fisheries Research Institute (CMFRI) being the pioneer to initiate open sea cage culture in Indian waters has been striving hard to promote open sea cage culture at selected locations in all the Maritime states with the involvement of the fisherman community. Cage

design and mooring technology has been undergoing refinement through the dedicated and committed efforts of the scientist of CMFRI. Efforts were continuously made to reduce the cost of the cage and mooring systems so as to make it affordable for the fisherman and also to help them to take it up as a lively hood alternative.

A high density polyethylene (HDPE) Cage of 6 m diameter costs about Rs.2,50,000/- and with the mooring systems and net, the cost increase to about Rs.3,50,000/- making it unaffordable to the fishermen and small entrepreneurs. On interacting with the fisherman they expressed to have a cage costing less then Rs. 1,00,000/- and lasting at least for 5 years to make it sustainable and economical in the long run. It was with their interest in mind the Karwar Research Centre has looked for alternatives for HDPE cages for promoting cage culture in the coastal waters and developed this fifth generation cage.

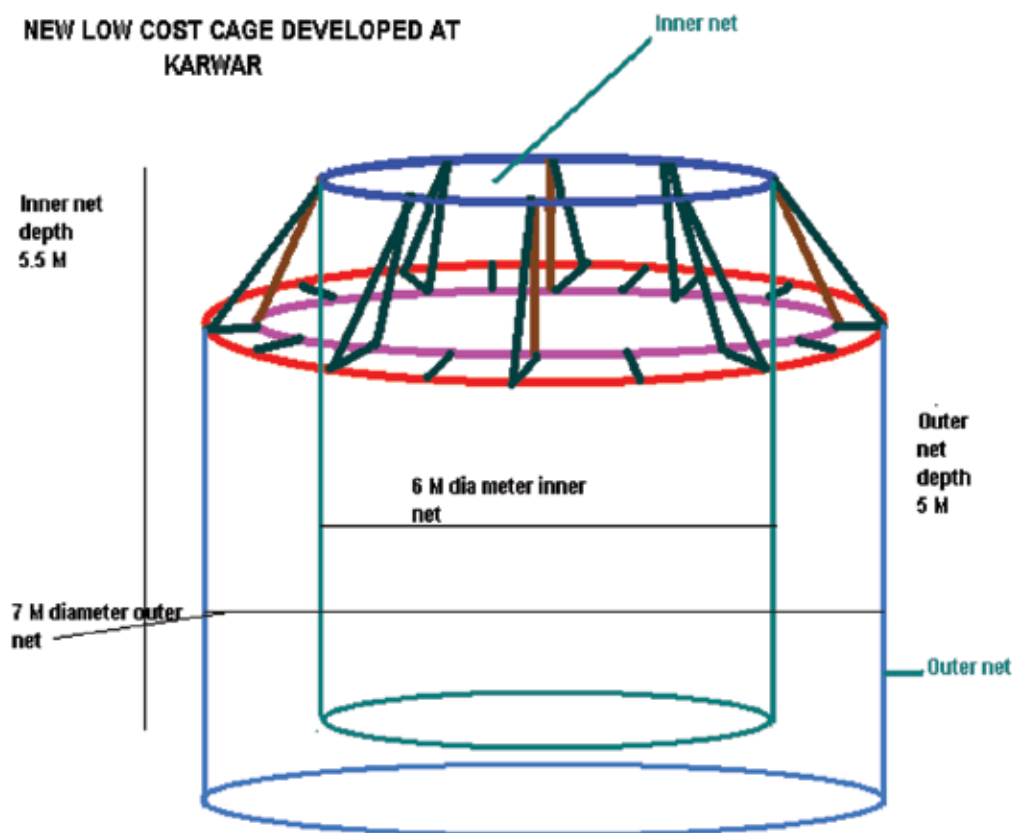


Fig.2. Technical details of the low cost cage



Fig.3. Low cost cage before epoxy coating

Design

The low cost cage developed at Karwar is made of 1.5" GI pipe (B class). The design details of the cage are given in Fig.1 & 2. The diameter of the cage was 6 meter and the height was 120 cm from Base to the railings (Fig.3). All the joints were double welded for ensuring extra strength (Fig. 4). After fabrication the cage frame was coated with single coat epoxy primer and double coat epoxy grey paint to prevent rusting. The total weight of the cage is about 300 kg.

Floataction

Puff or foam field HDPE cage is buoyant enough to float in the water. However, GI cage needs additional floatation and eight fiber barrels of 200 l capacity filled with 30 lb air was used for this purpose. The cage when floated on inflated barrels provides a stable platform around the cage where fishermen can stand and safely do net cleaning, net exchange etc (Fig.5).

Advantage of the low cost cage

The HDPE cages floats on water surface hence the outer net is always in the water level and predatory fishes enters into the area in between outer



Fig. 4. Welding details of the joints

and inner net. In the case of low cost cage the outer net is 60 cm above water level and provides no chance for predatory fishes to enter in the middle space.

HDPE cage sinks if more than three person climb on the side frame where as the low cost cage can take the weight of as many as 20-25 persons on the platform safely. The cost of 1 HDPE cage including netting, mooring etc, together costs about Rs. 5,50,000, whereas the low cost cage including netting, mooring all together cost only Rs. 1,00,000. The HDPE cage may take a

minimum 4 to 5 Crops to recover the input cost whereas low cost cage can recover the investment in a single crop itself. The diameter of the HDPE cage and low cost cage is 6 meters and Depth of the net also is 6 meters. Hence area wise both the cage gives the same performance.

Disadvantages

Unlike HDPE cage wind action is more on metal cage as it is floated on barrels. Hence it will be difficult to float in open sea condition from June to August unless Heavy duty mooring is provided. Except for this the metal cage performance is far superior to HDPE cage. For fabrication of HDPE cage costly parts and specially trained per-

sons are required. Hence fabrication charges are very high. Whereas for GI cage once the design is provided any small scale workshop can make it. HDPE cage once abandoned is an environmental hazard whereas GI cages once abandoned can be recycled.

Open sea cage culture in India is promoted by the government of India in a big way to increase fish production from coastal waters and to provide livelihood option to the fishermen. In this context CMFRI's initiative to reduce the cost of the cage to make it affordable to the common fishermen, will go a long way in resource and employment generation.



Fig. 5. Low cost cage in the farm



Fig. 6. GI cage provides an excellent working Platform for the farmers

Dismantling type Cages

GI cages reduce the cost of the cage by almost one fifth of the HDPE cage and increase the profitability of the operation. The whole concept of developing the low cost cage was to reduce the input cost and increase the profitability. The earlier GI cages were designed as fused cages where all the joints are welded. In such cages transportation of the cage was a problem and once the cage is welded it cannot be transported from one place to another by road. Another issue was that for the final welding of the cage power was not available at many places and hiring generator works out very costly. Another issue was that the water volume available inside the cage decides the number of fishes that we can grow in that. A six meter cage can hold 141 m³ volume of water for cultured stock. Another important observation was that all other expenses like mooring materials; floatation materials etc remain more or less same. Considering all this an attempt was made first to make the cage a dismantling type without affecting its strength.

Initially a 6 meter cage was designed and fabricated as dismantling type and tested it for strength, transportation efficiency and cost difference. When we found the design strong as a next step we designed a 10 m and later a 12 m



Fig.7. Assembling the 12 m cage in the beach

dismantling type circular GI cages. The volume of the 10 m cage is 392 m³ and that of the 12 m cage 565 m³. This innovation has increased the cage volume by 4 times and the production per cage to 21.6 tonnes (Table 2). Another advantage is that cages can be fabricated in small scale industries units where they get subsidized power and transport it anywhere by road. Similarly after the harvest the cage can be dismantled, serviced and stored in a shed and used again for the next farming when climate is favourable. 6 meter cage can be managed by 6 persons where as for the 12 m cage 10 persons are required. In short having one 12 m cage is like having 4 cages of 6 m diameter. So this path breaking design is going to make cage farming very profitable.



Fig.8. Dismantling type 10 m cage in the sea



Fig.9. Dismantling type 12 m cage in the sea

Table 1 Cost estimates of the GI Cages

Sl. No.	Material	6 m	10 m	12 m
1	GI Pipe	18900	37400	45900
2	Welding Charges	10000	20000	24500
5	Epoxy Paint	1600	2600	3600
6	Labour charges	1000	1500	2500
7	Floatation	7500	12000	13500
8	HDPE Rope	1000	1500	2000
	Total	40,000	75000	92000

Table 2 Production Capacity of GI cages of different diameter

Sl.No	Details	6 m	10 m	12 m
1	Cultivable Water Volume (m ³)	141 m ³	392 m ³	565 m ³
2	Stocking Density	5000	15000	20000
3	Sea Bass Production capacity in kg (60% survival rate and average weight 1.8 kg weight after 8 months grow out (Oct- May)	5400	16200	21600
4	Gross Revenue (Without deducting expenses) assuming that seabass fetches an average price of Rs.250/kg	Rs.13,50,000	Rs.40,50,000	Rs.54,00,000

Cage frame is the structure that holds the cage net safely throughout the culture operation in the sea. Since the cost of the cage nets mooring etc are same for any type of cages it is advantageous to go for a cost effective structure so that the input cost for the farming greatly decreases and profitability of the cage farming increases. GI cages are being used in Gujarat, Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu effectively. Low cost GI cages are playing a major role in popularizing open sea cage farming among the farmers and fishermen along the Indian coast catalyzing the growth of the blue revolution in the country.

Cage mooring systems

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Introduction

Moorings are required to keep the cages in a fixed position and to reduce the transfer of excessive forces generated by wind, currents and waves to cages. In well protected bays and seawater sites and freshwater sites, the forces exerted by environmental factors are reduced, small mooring system can be used. Open sea cages where cages are exposed to greater environmental forces require more effective mooring systems. Mooring depends on the type of cage, how exposed the sites are to weather, and the requirement for positional precision. Mooring failures were common place in the early days of coastal farming, but a better understanding of the problems, and more sophisticated analysis has largely reduced these risks. Cage and mooring design is “site specific”, and careful and combined choice of cage type, nets and most specifically moorings, has a considerable bearing on the ability of fish stocks to survive in major storms, on exposed sites.

The mooring system for a fish farm consists primarily of ropes, floats and anchors. In addition, several smaller components like shackles, connection plates, chains, rings etc. The moorings influence the stress acting on cage structural members and the behavior of the cages in rough weather, and can affect production, profitability and staff safety. They are therefore an important – indeed -, integral part of the cage system and should be carefully designed. Thus the collar, net and mooring components of a cage system should be designed together, although in practice the cages are usually chosen or built first with the mooring system being designed as an afterthought.

Moorings requirements should be determined by the design and type of the cages and the char-

acteristics of the site. It would first be necessary to quantify the incident forces that are likely to act on the cage under the worst possible weather conditions, and then to evaluate the proportion of energy transferred to the mooring lines and anchors. Two types of analysis can be used: quasi static and dynamic response. The loadings transferred to mooring lines vary enormously depending on current and wave conditions, cage design and number of lines employed.

Moorings design for a specific cage system and site

Wind and current forces are proportional to the square of the velocity. Thus an increase in current from 1 knot to 2 knots will generate 4 times the drag on a rigid submerged body. Effect of wave force is more difficult to compute, because the dynamic response of a system depends on so many factors. A change in the mooring system will change the internal loads on the cage system. In general a mooring system should be designed not only for specific cages, but also for the expected site conditions of water depth, wind, waves and current.

Moorings components

For a proper mooring system for any type of cage, a number of elements need to be assembled together, correctly specified and installed, physically and operationally compatible with each other, and effective in use and maintenance. Important components include the anchor or mooring unit on the seabed, the rising line, which connects the anchor to the surface system, and the surface or subsurface mooring grid. The major elements comprise several smaller sub-units – particularly links, shackles, droppers, safety lines, buoys, etc., which in effect are integral in the complete system.

Anchor specifications

A range of different types is available, commonly from the shipping/fishing industry. Major options are usually between gravity or dead weight devices – mooring blocks or mass anchors, which rely primarily on their weight, and those which rely on their ability to wedge into the seabed substrate. The holding coefficient of the anchor (k) is defined as (R) the horizontal force divided by the mass of the anchor. The holding coefficient (k) depends upon the angle between the anchor and the cage and thus the ratio between water depth and line length and the nature of the substrate.

The simplest and cheapest type of marine anchor is the dead weight or block anchor, which typically consists of a bag of sand or stones or a block of concrete or scrap metal. To prevent block anchors being displaced on the bottom, good friction between anchor and the bottom is necessary; this depends on the bottom condition and is given friction coefficient. Block anchors have low holding power per unit-installed weight. Block anchors are not recommended for use in rocky ground. Concrete block anchors may be simply fabricated using wooden shuttering, tyres or any other convenient object as mould. Steel rods for strengthening and eyebolt for a mooring attachment are usually incorporated. Once fabricated, the blocks can be transported to the waters edge at low tide and floats attached, so that they can be floated to the required location at high tide. Once installed, they are difficult to recover.

There are numerous types of embedding anchor. The holding power of an embedding anchor is related to its frictional resistance in soil, and so is dependant on fluke area, soil penetration and the mechanical properties of the soil rather than simply the mass of the anchor. They designed to be dragged down into the ground like a plough and become fixed. The holding capacity of drag anchor has been reported to be upto 25 times the weight with good bottom conditions. Embedding anchors are very efficient, i.e. they have a high holding power to mass ratio. Under optimum conditions, they are 10-500 times as efficient as block anchors. They are more expensive than block anchors in terms of cost per unit holding power and have to be bedded in properly. The use of two anchors connected together gives greater holding

power than the sum of independently moored anchors. There are numerous other type of anchor, combining the properties of block and embedding types, while others are designed for particular types of substrate.

Prior to choosing or installing anchors it is advisable to survey the sea bed. Anchors should be positioned first. The position of the anchors can be accurately established using a global positioning system or by taking bearings with respect to local. Easily visible land marks.

Rising line components

A range of materials and configurations may be used, the most common of which involves a chain section at the lower end of the line, a synthetic rope in the main upper length, and various elements of buoyancy or weighting to adjust the profile of the line, and its response geometry when subject to varying load. A range of different types and specifications may be available for chain and rope. Key issues concern weight and tensile strength, elasticity (length change with applied tension), stretching, dimensional wear, degradation. Float units need to be specified according to volume and shape, and to their resistance to deformation when submerged

Mooring lines must perform two functions: they must withstand and transmit forces. The loads imposed on a cage mooring system are principally dynamic. It is important that mooring lines have a high breaking strength and can absorb much of the kinetic energy of rapidly changing forces, otherwise these forces will be transmitted directly to the anchors. Natural fibre rope is not suitable as it is easily abraded and prone to rotting. Steel cable, although immensely strong, is expensive and heavy. Chain is extremely strong but again is heavy and is usually used in conjunction with synthetic fiber rope. Synthetic ropes of same diameter nylon and PES are considerably heavier than PP or PES. However, nylon is much stronger on a per unit weight or equivalent diameter basis than ropes fabricated from the other materials. Braided ropes are lighter than laid ropes and are generally weak. They also cost more and have few advantages other than they are easier and more pleasant to handle and do not kink. Although it can cost twice as much as PE or PP rope of equivalent strength, nylon has high extensibility and thus energy absorbing properties, an important factor in designing cage moorings.

Ropes should not be attached directly to either shore or sea anchors, but instead should be connected via a section of chain. The chain serves to increase the effectiveness of the mooring system, which directly act as an efficient type of anchor and improves the holding power of existing anchor by both reducing the angle between the mooring line and anchor and by increasing energy absorbing properties of the mooring line.

Moreover, a section of chain is necessary at the anchor since it is much resistant than synthetic fibre rope to the prevailing high abrasion forces. There are several types of chains are available. Wrought iron is very variable in quality; the best has excellent corrosion resistance while the poorer grades are inferior in all respects. Mild steel chain, with low carbon and manganese contents has been widely recommended for cage anchorages. A fairly heavy grade of chain is recommended.

The total length of the mooring line should be at least three times the maximum depth of water at the site and where the rope joins the chain, a galvanized heavy duty thimble should be spliced into the rope and a galvanized shackle of the appropriate size should be used to connect the chain and the rope.

An alternative mooring line composed mainly of chain is occasionally employed. Typically 12-25 mm chain, two or three times the maximum depth of water in length is connected from the anchor to a float positioned 10m or so from the cage and a section of rope –PES or nylon- used to link the floats to the cages. The buoy minimizes the vertical loading on the cages and must be sufficiently large to support the mass of the chain in the water and to resist the vertical forces imposed by the cages on the mooring system. Under shock loads, the chain/buoy acts as a spring absorbing much of the energy that would otherwise be transmitted to the anchor.

Two types of mooring systems be used: multiple and single point. The former is more common and involves securing the cages in one particular orientation while with the latter the cage are moored from one point only, allowing them to move in complete circle. Single point moorings tend to be used with rigid collar designs in sheltered sites. They use less chain and cable than multiple point moorings and because they adopt a

position of least resistance to the prevailing wind, wave and current forces, both inter cage forces and torsional forces at linkages are reduced. Single point mooring systems also reduce the enormous net deformation seen in conventional mooring systems and have been used with successes to moor large offshore cages. Cages moored from a single point also distributes wastes over considerably larger areas than those secured by a multiple point system.

The orientation of cages with multiple moorings depends upon the nature of the site and upon the type and group configuration of the cages. If particularly exposed or if currents are strong, then it may be best to secure cages in the position of least resistance to the prevailing wind and current forces. Where a site is sheltered and water circulation is poor, it may be better to moor cages so that water exchange is maximized. However, there may be restrictions on mooring orientation imposed by the site size or by suitability of mooring grounds.

The number of mooring lines used determines the distribution of forces to the anchors. Most methods of mooring involve the use of ropes and chain to link the cage or cage group to anchors or pegs secured to the sea bed. The mooring line is often termed as a 'riser'. Although this is most common system there are alternatives. Some cages may use a submerged rope or cable based mooring grid, to which cages may be attached temporarily using near horizontal lines. One further alternative is to drive long posts into substrate and to attach cage directly either with ropes or with metal hoops or tyres that permit some vertical tidal and wave induced movement. In theory the number and dimensions of posts required and the to depth to which they must be buried could be computed from the estimates of the forces acting on the cage system and data on the soil characteristics, but in practice it is determined by experience. Although sometimes employed in sheltered and shallow inland and coastal sites with suitable substrates, this method of mooring is not widely used.

Installation methods

The installation of mooring systems is an important aspect of the overall development of a cage site, and requires to be planned with care.

- i. Working base: a suitable and secure area for storing and laying out the mooring components needs to be identified – ideally a level, surfaced area.
- ii. Workboat or mooring vessel: capable of moving and positioning the mooring components and operating in the expected site conditions
- iii. Cranes: dockside and on mooring vessels – capable of lifting and moving the mooring elements safely at the required horizontal reach.
- iv. Access: – for materials to be taken to the assembly areas, for mooring components to be taken safely to the intended cage site.
- v. Marking out: key locations in the mooring site can be marked out on a hydrographic chart, checked on site with GPS or conventional optical surveys; local transect markers can be identified, and temporary positions marked with light lines and floats.
- vi. Making up moorings: the mooring lines and grids need to be adjusted to length and assembled to form the appropriate sub-components, which would then be finally linked together on site once the anchors are laid. Primary work can most easily be done on shore, using temporary measure lines or markers to help lay off the line lengths. Further adjustments can be done at sea, and all components and connections given a final check before installation.
- vii. Laying anchors and risers: if blocks are used, these can be set at the intended site, using positioning co-ordinates to define the location. For embedding anchors, these should be dropped a suitable distance outwards (i.e., opposite the direction of tension) from the place of intended location, and tensioned inwards to their final position. Laying of moorings and lines should be done carefully, taking particular care not to foul anchors with riser line, to tangle or snag the line, or to endanger staff.
- viii. Tensioning the rising lines: these need to be finally adjusted to ensure that the cage and/

or mooring assembly is correctly and evenly tensioned around its axes.

- ix. Diver swim of rising lines: finally, it is very important to check the whole system visually – to ensure that blocks or anchors are cleanly placed and/or embedded, that lines are lying properly and are not kinked or tangled, and that connections are sound.

Mooring maintenance

Cage moorings are a dynamic system, which must respond to motion, under load, every minute of the years it is installed. Maintenance is critical, to ensure that components are physically sound and that linkages are secure. Critical dimensions of items subject to wear – chain links, brackets, shackles, splicing eyes, need to be checked periodically, bolts and shackle pins need to be tightened, and riser lines may need to be adjusted.

With a rigorous and effective system of maintenance of both cages and moorings, with clearly defined parameters for replacement or repair, a well designed and installed system should be capable of reliable and secure operation.

Mooring systems must be checked at regular intervals and fouling removed from buoys and mooring lines. It is essential that any mooring inspection assesses component strength to see if it deviates significantly from design strength and that it should also assess likely deterioration in the interval to the next inspection.

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Nursery rearing and stocking of Asian seabass for cage culture

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Introduction

The increasing demand for fish in domestic and export markets indicate good prospects for large scale sea farming and coastal mariculture. The open sea cage culture has been expanding in recent years on a global basis and it is viewed by many stakeholders in the industry as the aquaculture system of the millennium. Cage culture has made possible the large-scale production of commercial finfish in many parts of the world and can be considered as the most efficient and economical way of rising fish. It is well known that availability of seed in adequate quantities is one of the major constraints in the development and expansion of mariculture. The increasing exploitation pressure on the wild stocks of many major marine fisheries has led to over exploitation and consequent decline in their catch and hence the only sunrise sector to augment seafood production is through marine farming.

Though seed production technologies have been developed for many marine finfish and shellfish species, many of these technologies have not been scaled up to commercially viable levels. The seed production of the commercially important species by development of viable technologies is essential for development of sustainable mariculture practices, however many of these technologies are still in the emerging state and may take several years for standardization on a cost effective way. The most common cultivable candidate species of marine finfishes include the rabbitfish (*Siganus* spp.), sea bass (*Lates calcarifer*), groupers (*Epinephelus* spp.), snappers (*Lutjanus* spp.) and sea breams (*Lethrinus* spp., *Sparus* spp.). Currently mariculture of finfishes is almost entirely supported from the seed collected from the wild, except for sea bass, pompano (*Trachinotus* spp.),

and cobia to some extent. The hatchery seed production of many high value marine finfishes and shellfishes is complex and expensive due to the high costs involved in the establishment of broodstock and hatchery facilities and also to the complicated larviculture procedures involving culture of proper live feeds, their nutritional enrichment, feeding protocols, grading, water quality maintenance, nursery rearing and disease management. From the available hatchery technologies and seed production techniques it is understood that the entire process, very complicated and diverse as broodstock development and spawning is very difficult and completely different from larval rearing and larviculture practices. The requirements as well as the techniques are extremely different and different sets of man power, techniques, and infrastructural facilities are required. Similarly the nursery rearing is another step in the seed production particularly if it is for cage culture. Nursery rearing can be done as the primary steps in case of pond culture in the same culture conditions; whereas in cage culture it is entirely different, it requires comparatively larger seeds in for culture as the fish culture in cages were in different culture conditions of open water systems.

Nursery rearing of Sea bass fry

Nursery rearing is usually carried out in three different systems; 1) tank systems, 2) in ponds 3) in hapas in ponds.

Tank: Nursery rearing of Asian seabass in indoor cement tanks was under taken. Immediately on arrival, the fishes were given a freshwater dip and placed in four cement tanks each of size 10' x 6' x 5' containing 7000 l of seawater (2500 fingerlings/ tank) with continuous aeration. The juveniles were reared in nursery rearing tanks upto 45 days before they were shifted to open sea

cages for further experiments. From second day onwards, the fish were fed commercial fish feed with a pellet size of 0.5 mm diameter at 4% of the body weight, four times a day (6.00 am, 12.00 pm, 6.00 pm and 12.00 am) for the first 15 days. Then the pellet size was increased to 1 mm for next 15 days while the feeding rate and frequency remained unchanged. For the remaining 15 days, the fishes were fed with a pellet size of 2 mm. Eighty per cent of the water was replaced 5 min after feeding with 20 min flow-through thereafter. It was confirmed that the feed was consumed immediately after feeding with no visible feed pellets settled at the bottom. The fishes were graded every 15 days with an automatic grader and grouped into three different sizes. After grading, representative samples were collected for studying growth parameters.

In Floating net cages: Floating net cages system is used in China where net cages are hung on wooden rafts and kept afloat with cylindrical plastic containers or Styrofoam. The average net cage is 3x3x3 m, and depending on the size of the raft, 6-16 cages are secured together per raft. The cage unit is stabilized with zinc-placed pipe and anchored to the bottom. During the nursery period, size grading should be conducted every 15 days to avoid cannibalism. At the same time, the net cages should be checked for damage to insure that fish do not escape. In the early stage of net cage culture, fishermen collect 1-2.5 cm fry from the wild and stock them for one to two months at a density of 2,000 seeds/net cage. Beyond the nursery period, juveniles are size graded and stocked into separate grow out net cages. It has been recorded that in terms of growth, survival and economic results, nursery reared juveniles perform better than those stocked directly into cages.

In hapa in Pond: The hapa usually used are 2x1x1.5 m and are usually set in open waters one day before stocking to remove the contaminants if any. Stock of 2,000-3,000 fry are raised to the fingerling size in these cages. Two sets nursery rearing trials comprising 5 to 6 hapa each were carried out in two different locations for cage culture works. In the first set the fries were small ranges from 15 to 20 mm in length were stocked in a stocking density of 1500 to 2000/ hapa and fed with live feeds like mysids and small prawns for initial two weeks and gradually changed to

chopped fish and prawns. The growth was very fast and growth difference was high at this stage and therefore grading was done every fourth day. The survival was around 90% and cannibalism was very less. After two weeks the feed changed to chopped fishes/prawns. During this stage also very good growth and survival was observed. However there were little problems due to low water quality especially in the early morning hours. The excess feeds caused high algal growths in the ponds and that led to the lower oxygen levels in the pond water during early morning hours. This was mainly due to the water exchange in the pond where the hapa was installed was less. This could be controlled by providing additional aeration to the hapa. The excess feeds remained in the hapa attracted crabs towards the hapa were damaged as the crabs bites the nets. The rearing was done for a period of 45 days and the fries reached 8 to 12 cm in size with an average body weight of 60 g were transferred the cage.

In the second trial the initial size of the fries varied from 25 to 30 mm in length. So feeding started with chopped fish/shrimps. The hapa were installed in a pond with good water exchange. Here also cannibalism was negligible and survival was very good (90 %). The growth was encouraging and fishes reached a size of 10 to 15 cm in three months with a body weight of 90-120 g with three months. Here also 6 to 7 hapa were used at a time; were fixed to poles at corners and tied in parallel row as shown in the figure. Grading was done in every 3rd day by hand picking. The growth difference was comparatively less compared to the first set of experiments may be due to the good water quality.

Salinity acclimatization: Seabass is a euryhaline species except in its early larval stages. The fish can be easily acclimatized from sea water to fresh water within short periods of time without any mortality. It can easily adjust 5-10 ppm at a time and again it can be changed after a period of 1 hr. Like that with one day itself it can be changed from sea water to fresh water and vice versa.

Collection and Conditioning of Fry before Transport: Fry are collected from the rearing tanks and placed in smaller receptacles. Fry are treated with 5 ppm of acriflavine solution or 0.5 ppm of copper sulfate solution for 5-10 minutes. There should be no feeding within 1-2 hours be-

fore packing. Plastic bags of 40 × 60 cm of proper gauge are filled with 6–7 litres of fresh seawater and saturated with oxygen; 10–12 litres of oxygen gas are used for packing. The amount of transportable fry depends on size of fry, water temperature in plastic bags and duration of travel and handling from source of fry to its destination.

Transport: In transporting by truck, a mixture of crushed ice and sawdust is needed to control the water temperature in the plastic bags during

transport. The mixture is spread uniformly on the floor of the truck before the plastic bags are laid upon it. The proportion of crushed ice and sawdust is 1:1 for long period transport (12-16 h) and 1:2 for short periods (4-5 h). Transportation should be carried out at night time. By this method, it is possible to control the water temperature between 19–23° C. Transport for short distances can be done with the aerators in tanks in low temperature at a density of 250 juveniles/tonne water up to 5 -6 h.

Grow-out culture of finfishes and management of marine cages

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Introduction

The biology and culture requirements of each species determine the production from any culture systems. A candidate species for aquaculture is determined based on different parameters which affect production. The important grow-out phases are the initial nursery and later grow-out phases. For hatchery produced seeds which are hardly 30-45 days old, nursery phase of fry is very crucial and needs special care.

Nursery rearing of fry

In nursery stage, the fry are reared to stockable size which is called the fingerling. Nursery can be carried out in tanks, ponds or in net hapas. In either case, adequate water exchange is essential to maintain optimum water quality. Nursery ponds may range in size from 500-2000 m² with a water depth of 1-1.5 m is considered. Optimal stocking density should be arrived on the basis of environmental requirements of a given fish species and economic efficiency. Daily at least 30 % water exchange is required in the pond. Fry must be fed with supplementary feed of good quality and the feeding rate is adjusted depending on the species reared in the nursery. Some species mainly carnivores require grading (removal of shooter fish) in every 3-4 days to reduce cannibalism. The survival rate in the nursery phase can be up to 90 % in 30-45 days of rearing. Hapa should be checked and cleaned regularly. The fry on reaching a size of 25-40 g at the end of another 30-45 days can be stocked in the open sea cages for the grow-out system. The survival rate for the nursery period would be 50 to 80 %. This would depend on feeding, environmental conditions, and the skill of the fish farmers.

Grading of fry and transport of juveniles: Due to cannibalistic nature, for fish like seabass, size

selection or grading or sorting of the fry is important. Grading is initiated while in the hatchery itself at around 12-15 days old, and done every 3-5 days. Mechanical or manual grading is followed. Different types of graders fixed as well as adjustable types are now available in the international market. Since the number of fishes in the tanks as well as in the hapa is high, the competition for feeding increases and the fittest will only get the feed and this makes it essential to grade the fish more frequently. For Asian seabass grading is essential at least till the fish reach 30-40 g, which is ideal for stocking in grow-out cages.

Fingerlings of 25-30 g or even more are starved 2-3 hours prior to transportation to grow-out systems. If the salinity of grow out is different, the fishes are acclimatized to the same salinity prior to transportation and if required minor sedation can be provided for bigger fishes.

Stocking density and production

The stocking densities of species in cages are highly variable and the optimum density for many species is not known. Stocking in cages can be high density/ low volume or low density/ high volume. Low density/ high volume stocking (2-20 fish/m³) are recommended for species like Cobia for which harvest size is several kilograms; high density/ low volume stocking (50-250 nos/m³) are good for those fishes target harvest is 1 kg or less. The number of fish to be stocked also depends on the carrying capacity of the cages. The stocking in a cage is done keeping in mind the production target in the open water. The ideal stocking is to produce 25 kg/m³. However, there were several instances where production was enhanced to 100 kg/m³ in open sea cages. Optimal stocking rate ensures optimum yield at the end of the culture

period as food conversion, survival rate, and condition of the cultured fish are closely related. The smallest recommended fingerling size for stocking is 15 g. A 15 g fish will be retained by a 13 mm mesh net. Survival rates in well-placed and well-managed cages are typically 98 to 100%. Optimal stocking rate varies with species, feeding behavior and environmental characteristics.

Feed and feeding

For aquaculture, the most important factor is feed and feeding. A cost-effective feed of good quality and acceptance determines the production in any culture system. The nutritional requirement is dependent on the cultured species and its stage of development. Young fish are fed with high protein diet which is reduced with age. Carnivores like seabass and cobia are fed with high protein diet all through the culture duration. To arrive at a cost effective diet the nutritional requirements of the species has to be known and accordingly the feed has to be formulated. Feeding is a vital operational function and may include many biological, climatic, water quality, and economic considerations. The direct influence on growth rate in terms of feeding intensity, feeding time, food rations are important economic considerations a farm operator has to make. The feeding characteristics of each species vary in maximum feed intake, digestion, feeding frequency and conversion efficiency. These in turn have direct effects on the net-yield, survival rates, size of fish and the overall production.

Cage management

The growth and thereafter production can be regulated through proper management of the various culture activities. The major factors to be taken care in cage management are:

- Stocking the species at optimum density appropriate to the site and rearing conditions
- Feeding in cost-effective manner aimed at maximum production
- Best possible water quality within the cages
- Periodic maintenance of the cages, moorings, anchors, nets and related accessories
- Regular monitoring of the stock by sampling, for health conditions or any abnormal behaviors
- Grading is essential during the initial stage of growth for cannibalistic species,
- Water quality monitoring on a regular basis
- Record keeping on water quality conditions, growth and mortalities

Feed management

Feed and the feeding regimes need proper management for better health and growth of the cultured stock. However, the quality and safety of feed and the use of fish medicines and chemicals must be controlled by concerned agencies so that it will integrate aquatic product security examination, environmental monitoring and fish disease prophylactic systems at different levels (Imelda-Joseph, 2009). Storage facilities are essential for cage fish farming operations. Feed bags should be stored without open access to moisture to prevent fungal attack. Trash fish may arrive at the farm in either frozen or unfrozen state and since fish spoils rapidly it should be checked for freshness before being stored. Smell and appearance should be sufficient indicators of quality. Cold storage is ideal for trash fish. Feeding should be done throughout the culture period at varying levels depending on the growth rate and natural feed availability. Hand feeding is done in most cases and is recommended for small scale farmers. However, mechanical feeders are used in large scale cage farms- demand feeders and automatic feeders are the two types of feeders used. Feeding trays or feeding rings can be used based on the type of feed used.

Water quality management

Routine monitoring of water quality is essential

- To avoid losses caused by lethal changes in water quality
- To evaluate site and configuration of cage
- To maintain optimum stocking and feeding requirements
- To evaluate the general condition of stock, so that if stressed, can avoid handling.
- To gain information of long term changes in water quality at a site so that variation in production may be properly evaluated.

Waste control and effluent management

Cage-farm wastes are usually in the form of uneaten feed and faeces. Feeding should be scheduled in such a way to ensure that feed wastage is kept to a minimum. Use of floating feed is vital for cage-farm operations. Mooring cages in deep waters, leaving 3-5 m bottom space and where good current flow results in cage wastes being easily flushed away, thereby avoiding organic build up under the cages.

Health management practices

Health management is essential to maintain a good health status, assuring optimum productivity and the avoidance of diseases. In aquaculture, the economic risk associated with diseases is high and represents a potential loss in production through mortality and morbidity. Moreover, the cost to treat diseases when they are already well established is high and treatments are often initiated too late and are therefore rarely effective. Thus, aquatic animal health management must be a global strategy that aims to prevent diseases before they occur.

Aspects of fish health management

- Responsible transportation of live aquatic animals
- Hygiene, disinfection and biosecurity
- Selection of hatchery-raised fingerlings
- Record keeping and disease monitoring
- Proper disease diagnosis

Harvesting

Harvesting in a cage culture system is a simple process. Harvesting can be done in a single lot or in batches based on demand and market price. For flexible cages the net can be lifted and the cultured fish collected by means of a simple scoop net. For rigid cages, the cage has to be lifted to facilitate harvesting. No sophisticated harvesting technology is required. In most marine cage culture practices, the harvested fish are kept alive such as yellowtail, breams, snappers and groupers and transported immediately to the markets or restaurants. Preservation and processing of cul-

tured fish will be an essential part of the culture industry when aquaculture is further developed.

Maintenance of cages and gear

Irrespective of the damage that can be caused by storms, predators, drifting objects, poachers, all materials used in construction of cages have a definite life span and will eventually wear out. Cages, nets and moorings therefore must be checked at intervals for signs of damage and wear and tear and repaired or replaced if necessary, as not only cages and stock be put at risk, through neglect, but human life may also be endangered. Mooring must be checked regularly by divers, particularly after heavy wind/storms. Mooring level should be kept free from fouling and worn shackles replaced.

Cage nets may be checked during cleaning, which is done more frequently during cage culture. Divers may have to go down and observe the net every week or so, during favourable weather conditions. Small tears may be repaired at the site itself, while major repairs should be done on shore only. Net exchange has to be done periodically to avoid fouling constraints and good water exchange.

In marine environment fouling is a major issue and in rotating design (single point mooring system) it is reduced. Therefore, the nets have to be frequently changed. In any case, nets of any particular mesh size should be exchanged quite often for ones with larger size as the fish grow. Mesh size should be carefully selected at each stage of growth too. If too small mesh size is selected, then matter exchange is restricted and if too large, escape is possible. The frequency of net change varies from once in a week to once in a year depending upon the site location, materials used, season and management and design of cage.

Net cleaning can be done physically or by chemical treatment. Physical cleaning involves removing and scrubbing the net and drying. For chemical cleaning bleaching powder or formic acid (3%) can be used. The rate of bio-fouling on cage frame is much slower than on net cages, and doesn't need more frequent cleaning. Cage frames are usually cleaned in situ using a hand brush both above and below the water line to dislodge weed and accumulated debris.

Genetics in Aquaculture - A brief overview

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Fisheries and aquaculture sectors play a pivotal role in providing nutritional and livelihood security for the growing global populations. Fish genetic resources for food still come from the capture due to low domestication level in fisheries sector. Contribution of worldwide aquaculture to fish production has increased from 2.2% to more than 38% in 50 years. The aquaculture technologies so far developed are primarily based on husbandry and management practices. They have their own limitations and beyond which they may not be economical. At this juncture, recent technological advancements in the field of genetics and biotechnology have provided immense scope for the growth in aquaculture to meet the challenges ahead. The different approaches available in the field of genetics in fishes are the traditional selective breeding and hybridization, genome manipulations and hormonal sex reversal and gene transfer. The method to be applied usually depends on the species, its biological traits and its economic importance. Selective breeding has been proved to be an effective method to improve a number of quantitative or economic traits in a cumulative manner, i.e. an improvement from generation to generation.

Selective breeding

Selection is aimed at modifying the genetic structure of a breed in order to obtain animals with superior performances for the traits of interest. Selection may be done to increase yield, survival rates, and resistance to biotic and abiotic stress and also for improving product quality. Selective breeding that exists in nature is called 'natural selection'. In this process, the most strong and fittest individual that can withstand variation or the changing situations in their environment alone will survive. Such individuals which can

perform well or the best can also be developed through artificial selection also. Artificial selection is a classical approach and the methods have been profitably utilized in aquaculture too.

There are different methods of selection, viz., mass selection, family selection, within family selection, combined selection etc. In the case of fishes, most commonly followed methods are either family selection or mass selection. Most of the time, combined selection is also followed. While mass selection can be used for only a single trait, family selection/combined selection helps in selecting for different traits such as growth, disease resistance, meat quality or reduced fat content etc. In any selective breeding programme the individuals that perform more than the population average for the target traits are captive bred and progeny is again selected for generations for the breeding purpose. In the whole selection programme, caution is taken to avoid breeding of closely related individual which depress the possible genes due to inbreeding. In aquaculture, genetic selection and hybridization have been used for growth, carcass composition and quality. Some of the successful examples of selective breeding programmes in aquaculture are Atlantic salmon in Norway, Nile tilapia in Asia, Channel catfish in the USA. GIFT or Genetically Improved Farmed Tilapia, grows 60 per cent faster than the parental strain, is one of the widely used improved fish in tropical aquaculture. The other important aquaculture genetic improvement programmes that are in progress include *Cyprinus carpio*, *Labeo rohita*, *Barbodes gonionotus*, *Megalobrama amblycephala*, *Penaeus monodon*, *Marsupenaeus japonicus*, *Fenneropenaeus chinensis*, *Litopenaeus vannamei* and *Macrobrachium rosenbergii* for traits such as fast growth rate and disease resistance; and for smaller naupliar size

(SNS) in *Artemia franciscana* and Indian strains of *Artemia*. The work on selective breeding of the Indian major carp, rohu at Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar has resulted in the development of strains that exhibited over 40% selection response on an average at 4th generation level. Genetic improvement of rohu (Jayanti) with 17% higher growth efficiency per generation is reported.

Marker Assisted Selection (MAS)

MAS aims to choose the genetically superior individuals using molecular information. To perform MAS, markers tightly linked to the loci responsible for quantitative traits (QTL) or major genes directly involved in the phenotypic expression should be found. Linkage maps have been used for quantitative trait locus analysis and Marker Assisted Selection (MAS). Microsatellite DNA markers and Single nucleotide polymorphisms (SNPs) have become markers of choice for development of linkage maps. In MAS, identified polymorphic markers are used to genotype the families and progenies with desired trait data. Application of marker assisted selection is pursued in some aquaculture species such as catfish (563 markers, AFLP, with 43 linkage Groups); tilapia (550 microsatellite and 15 genes), sea bass (163 microsatellite and 25 linkage groups) and tiger prawn (189 markers with 36 linkage groups). In India, a programme to develop linkage map of *Labeo rohita* is being actively pursued.

Hybridization

It is well known that hybridization refers to crossbreeding between, either members of different races or strains of the species (intra - specific) or between two species of the same genus (inter - specific) and between species belonging to different genera (inter - generic). Hybridization is usually aimed to combine the positive traits of the parent species in their hybrid offspring. The positive traits may include better growth, resistance to disease/changed environment, meat quality, early or late maturity, better fecundity and so on.

Intra-specific hybridization: Different races or strains of the same species from different geographical regions when crossed have resulted in the production of offspring with heterosis. The most noteworthy is the interracial crossbreeding work in the erstwhile USSR between cultivated

common carp and the wild carp from river Amur. The offspring of this cross known as 'Kursk carp' has a greater resistance to cold temperature than the cultivated carp. This facilitated extending fish culture to the north and northeastern region of the USSR, which was hitherto nonexistent.

Inter - specific and Inter - generic hybridization: It involves hybridization between species or genus. Inter-specific and inter - generic hybridization work among Indian major carps, tilapia, sturgeons etc. has shown intermediate traits in some cases and negative traits in others.

In general, hybridization may be useful to combine the positive traits of the parents in the offspring or in producing monosex progeny and also sometimes changing the ploidy status, leading to sterile individuals in certain cases. The resultant traits in the hybrid offspring of any particular cross may probably depend on the compatibility and interaction between the genomes of the two species involved in the cross.

Hormonal sex reversal

Administration of exogenous steroid hormones to control sex in fishes has been in use for aquaculture purposes. The hormonal sex reversal techniques are used for the mass production of either sterile monosex population by interfering with the genetic sex. They are treated at an early stage (spawn or fry) with androgens (male hormone) if all male population is required or with estrogens for all female population. The spawn/fry treated with androgen usually develop testes and those treated with estrogen develop ovaries. Hormone administration is done either by mixing the required quantity in the feed or in dissolving in water medium (dip-treatment or immersion). The early stage of the fish for treatment should correspond with the initial genetic sex or gonadal differentiation. The success or effectiveness of hormonal sex reversal depends particularly on the time of treatment, dose of hormone, duration of treatment and also sometimes the mode of treatment. In addition to producing monosex fish populations, hormone treatments are also carried out to produce sterile fish. The most commonly used hormones for sterility are testosterone and methyl testosterone, at a dose slightly higher than used for masculinization. Hormone treatments have been successfully used to sterilize genetically improved tilapia (through selective breeding) to

prevent unauthorized production and sale of the seed of these improved varieties.

Male and female sex hormones: Androgens, both natural and synthetic are used for the production of all male fish population. Among androgens 17 α methyl testosterone (MT) is the most widely used hormone. Production of 100 percent male tilapia had been done by administering a dose of 5 mg MT/kg diet during the labile period lasting from 9 to 20 days after hatching. Estradiol 17 β and estrone are the two naturally occurring steroids, used to achieve feminization in fishes.

Negative aspects of hormonal sex reversal: Some of the expected and suspected negative aspects of hormonal sex reversal are i) the residues of the administered steroids can be carcinogenic and may interfere with the sex of consumers of the treated fish ii) sexual maturity in the sex reversed female is usually delayed and they may have reduced fecundity iii) it is a costly and time consuming process iv) the process has to be repeated every time whenever monosex/sterile population are required. In conclusion, though it is claimed with experimental proof that the hormone from the body system of the treated fish will be disappearing soon after the treatment is suspended, use of hormonal manipulation has to be carried out with necessary caution.

Polyploidy:

An individual is said to be polyploidy if its ploidy consists of additional set(s) of chromosomes over the normal diploid number

Natural Polyploidy: Natural polyploidy has been observed in some fish species like the common carp, trout etc. mainly due to chromosomal translocation. It may also appear in the cross bred progeny of very distantly related species. Thus, spontaneous triploidy was observed in the progeny of the cross between grass carp and big head carp.

Induced polyploidy: Polyploidy can be induced artificially in the same manner as artificial gynogenesis and androgenesis. In other words, shock treatments of the same nature and intensity and duration applied for inducing gynogenesis and androgenesis, to a given species may be also effective to induce polyploidy. The difference is that to induce polyploidy, shock treatments is administered to normal fertilized zygotes and not to genetically denatured sperm activated zygotes.

However, while triploidy (3n) is induced through the retention of second polar body in the fertilized egg by giving early shock treatments, tetraploidy (4n) is induced by blocking the first cleavage or mitotic division in the fertilized egg by administering late shock treatments (Fig.3). The CMFRI, Cochin has been successful recently in producing triploid edible oyster, *Crassostrea madrasensis* by treating the newly fertilized eggs with 100 μ M 6- dimethylaminopurine. This yielded 67% survival and triploid oysters exhibited significantly higher growth rate and meat content (126% higher dry weight, nearly 30% more glycogen, lipids and proteins) compared to diploid individuals. Triploids, particularly aneuploid individuals are generally sterile. Sterility can be made use of in species like common carp and tilapia which bred prolifically even under pond environment, to check unwanted reproduction leading to over-population that will hamper the growth of other fishes in the grow-out culture system for want of food and space. In some species sterile triploids have been reported to grow significantly faster than the normal diploids. Induction of tetraploids resulted in heavy mortality, poor yield and survival.

Genome Manipulation (Chromosomal Engineering)

Genome manipulation or chromosomal engineering is another modern approach to produce gynogenetic and androgenetic inbred lines and polyploidy individuals. Gynogenesis and androgenesis are effective tools to produce highly inbred homozygous lines of usually mono sex individuals, female and males respectively. These inbred individuals when crossed with normal heterozygous ones may produce offspring with heterosis, particularly for growth.

Gynogenesis:

The phenomenon of gynogenesis is generally stated to be a specialized form of parthenogenesis. In gynogenesis the embryo develops solely with maternal genome where in the egg is activated by the sperm without any genetic contribution from the latter. But then the resulting zygotes are haploid. Restoration of diploidy may occur spontaneously in the case of natural gynogenesis or by chemical or a variety of thermal/pressure shock treatments to the activated eggs in artificial gynogenesis.

Natural gynogenesis: Natural gynogenesis occurs in nature. In some species of fish of the family Poeciliidae such as *Poecilia formosa* and Cyprinidae (*Carassius auratus gibelio*), gynogenesis is the method of reproduction. Natural gynogenesis has been reported among the members of the family Pleuronectidae too.

Induced gynogenesis: During recent past, gynogenesis has been successfully induced in a number of fishes including Indian major carps and Chinese carps by denaturing the genetic maternal (DNA) of the sperm through irradiation either by UV or gamma rays and activating the eggs with the irradiated milt. Diploidy is restored as mentioned earlier. In some species diploidization of the activated eggs was effective with cold shock treatments while in others heat shocks gave better results. Among Indian major carps, diploidization of activated eggs was found to be better with heat shocks in catla and cold shocks in rohu and kalbasu. However, pressure shocks were reported to yield higher percentage of diploid gynogens in many species. Gynogenesis usually results in the production of all female progeny when the female is homogamous, means female fish that produce eggs having only x/x and not x/y eggs. Most of the females in fishes produce homogamous eggs. Gynogenesis is of two types that can be induced in fishes. One is meiotic gynogenesis and the other is mitotic gynogenesis. These two types of gynogenesis are effective tools to produce inbred lines of fish, in a much shorter time when compared to the conventional means of sib-mating which may take 10-12 generations to achieve complete homozygosity.

- i. **Meiotic gynogenesis:** Meiotic gynogenesis is induced through the retention of second polar body by administering early shock treatments to the activated eggs.
- ii. **Mitotic gynogenesis:** Mitotic gynogenesis is induced by blocking the first cleavage / the first mitotic division (endomitosis) by administering late shock treatments

Hormonal sex reversal is also used for the production of gynogen males by administering androgens to a portion of meiotic gynogen offspring. These sex reversed individuals are phenotypically males as they develop testes, but genotypically females as they produce sperm with X chromosome, so that when a gynogen female is crossed with this sex reversed gynogen male, the resultant offspring will all be females.

Androgenesis:

Androgenesis is also another form of parthenogenesis. It occurs naturally or be induced.

Natural or spontaneous androgenesis: Natural androgenesis though rare, has been found to occur particularly in some hybrid crosses, produced either between distantly related individuals or those with disproportionate or incompatible genomes as in the cross of common carp female and grass carp male. However, the incidence or percentage of occurrence is very rare and low.

Artificial androgenesis: In induction of artificial androgenesis, the genetically inactivated egg is activated by normal sperm of the species. Diploidization of the zygote is achieved through the administration of shock treatments as done in the case of artificial gynogenesis.

Population genomics-The future prospects

Population genomics is becoming an increasingly popular approach to identify the architecture of genes controlling various commercially important traits and their variations among populations at the target gene-rich regions of genome with the deployment of next generation of genome scans and strong bioinformation tools, and it is expected to play a major role in future selective breeding and aquaculture programs. The potential of molecular marker assisted selection and the domestication programmes should be further explored, benefitting from the development of new genome resources and analytical tools.

Biotechnological interventions in marine finfish breeding and seed production

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Introduction

Fish farming is the world's fastest growing sector of agricultural business. Fish and other aquatic products represent an important, underutilized resource for a protein-hungry world. Natural catches are dropping even as demand continues to increase, making aquaculture increasingly attractive. Consumer demand for fish products is increasing. At the same time, wild fish stocks are rapidly declining, mainly because of over-fishing. Biotechnology provides powerful tools for the sustainable development of aquaculture, fisheries, as well as the food industry. Increased public demand for seafood and decreasing natural marine habitats have encouraged scientists to study ways that biotechnology can increase the production of marine food products, and making aquaculture as a growing field of animal research.

In general, biotechnology can be broadly defined as using living organisms or their products for commercial purposes. As such, biotechnology has been practiced by human society since the beginning of recorded history in such activities as baking bread, brewing alcoholic beverages, or breeding food crops or domestic animals. Biotechnology allows scientists to identify and combine traits in fish and shellfish to increase productivity and improve quality. Scientists are investigating genes that will increase production of natural fish growth factors as well as the natural defence compounds marine organisms use to fight microbial infections. Modern biotechnology is already making important contributions and poses significant challenges to aquaculture and fisheries development. It perceives that modern biotechnologies should be used as adjuncts to and not as substitutes for conventional technologies in solv-

ing problems, and that their application should be need-driven rather than technology-driven.

The use of modern biotechnology to enhance production of aquatic species holds great potential not only to meet demand but also to improve aquaculture. Modern biotechnology is concerned with the use and manipulation of the DNA molecules, the genetic code, of living organisms. The emergence of modern biotechnology began with the discovery of a heat stable DNA polymerase enzyme, which produces many copies of small amounts of DNA in the Polymerase Chain Reaction (PCR). With this technique, selected proportions of the genome, including genes, can be rapidly amplified to amounts suitable for further analysis. Once the DNA sequence is available, the challenge is to use this new information in the genetic improvement of aquaculture species.

Genetic modification and biotechnology also holds tremendous potential to improve the quality and quantity of fish reared in aquaculture. There is a growing demand for aquaculture; biotechnology can help to meet this demand. Biotech aquaculture also offers environmental benefits. When appropriately integrated with other technologies for the production of food, agricultural products and services, biotechnology can be of significant assistance in meeting the needs of an expanding and increasingly urbanized population in the next millennium. Successful development and application of biotechnology are possible only when a broad research and knowledge base in the biology, variation, breeding, agronomy, physiology, pathology, biochemistry and genetics of the manipulated organism exists. Benefits offered by the new technologies cannot be fulfilled without a continued commitment to basic research. Biotechnological programmes must be fully inte-

grated into a research background and cannot be taken out of context if they are to succeed.

The two areas of modern biotechnology, which will probably have the most significant impact on genetic improvement of aquaculture species, are DNA markers and transgenics. One can safely say that genetic modification is as old as agriculture, at least. As long as man has carried out selective breeding of domestic plants and animals, we have also altered the genetic composition of organisms. Still, it is not too hard to see that there are marked differences between traditional 'old fashioned' breeding techniques and direct transferral of genes from other species. The benefits from producing GMO's are overwhelming in some areas: non-polluting enzyme-based detergents are produced by transgenic microorganisms; genetically engineered yeasts produce human insulin for treatment of diabetics; and bacteria-carrying human genes produce pituitary hormones for improving the lives of people with defective glands.

Aquaculture/ Mariculture area continues to excite particular interest in many forums. Since we know little about how to raise, protect and reproduce most species (except carp, tilapia and a few others), biotechnology techniques could help:

- Clarify the biology and life cycle of new species;
- Promote fertility, growth, meatiness and egg-laying through hormonal treatment;
- Diagnose fish diseases (common in overcrowded ponds) and prevent them with vaccines; and
- Promote the scientific raising of rotifers and other fingerling food.

One important commercial goal could be the economical production of disease-free embryos and fingerlings of food and ornamental fishes.

In global scenario, the fisheries and aquaculture is an important sector of food production, providing nutritional security to the food basket, contributing to the agricultural exports and engaging millions of people in different activities. In this similar line, the marine aquaculture or mariculture using biotechnological interventions has immense potential being a lucrative sector worldwide. The potential area of biotechnology in mariculture include the use of synthetic hormones in induced breeding, transgenic fish, chro-

mosome engineering (uniparental and polyploidy population), cryopreservation and gene banking, marker assisted genetic improvement and health management.

Induced breeding of fish

Gonadotropin releasing hormone (GnRH) is now the best available biotechnological tool for the induced breeding of fish. GnRH is the key regulator and central initiator of reproductive cascade in all vertebrates (Bhattacharya et al., 2002). It is a decapeptide and was first isolated from pig and sheep hypothalamus with the ability to induce pituitary release of luteinising hormone (LH) and follicle stimulating hormone (FSH). Since then only one form of GnRH has been identified in most placental mammals including human beings as the sole neuropeptide causing the release of LH and FSH. However, in non mammalian species (except guinea pig) twelve GnRH variants have now been structurally elucidated, among them seven or eight different forms have been isolated from fish species (Halderet al., 1991). The most recent GnRH purified and characterized was by Carolsfeld et al., (2000). Depending on the structural variant and their biological activities, number of chemical analogues have been prepared and one of them is salmon GnRH analogue profusely used now in fish breeding and marked commercially under the name of 'Ovaprim' throughout the world. The induced breeding of fish is now successfully achieved by development of GnRH technology.

Transgenesis

Transgenesis or transgenics may be defined as the introduction of exogenous gene/ DNA into host genome resulting in its stable maintenance, transmission and expression. The technology offers an excellent opportunity for modifying or improving the genetic traits of commercially important fishers, molluscs and crustaceans for aquaculture. The idea of producing transgenic animals became popular when the first produced transgenic mouse by introducing metallothionein in human growth hormone fusion gene (mT-hGH) into mouse egg, resulting in dramatic increase in growth. This triggered a series of attempts on gene transfer in economically important animals including fish.

The high fecundity of most fish and external fertilisation and embryonic development make

them especially suitable for transferring specific genes. Successful gene transfer has been demonstrated into a variety of aquatic organisms by applying different techniques, including micro-injection, particle gun bombardment, and electroporation. The transfer of genes into the sperm or directly into the skeletal muscle has become alternatives to the fertilized eggs. The production of transgenic fish is aimed at dramatic improving traits like growth, disease resistance, and environmental tolerance. The nature itself of such quantitative traits, being influenced by multiple genes, makes them difficult to manipulate by gene transfer techniques. It should be noted that the increased growth rate in the transgenic fish is mainly due to the great amounts of growth hormone produced in large tissues like the liver or gonads, and not the result of injecting millions of gene copies into the fertilized eggs.

There are several problems to be overcome before transgenic animals can be produced on a large scale. Indeed, in over 90 % of the microinjected eggs, the transgene is not efficiently integrated in the genome at the one-cell stage. The result is highly mosaic transgenic fish and low frequencies of germ line transmission since only the tissues developing from the transformed cell will carry the transgene. Furthermore, the injected DNA integrates at single or multiple random sites in the genome of the recipient embryo, each developing into a unique hemizygous fish. Hence, the establishment of a stable transgenic broodstock will be a costly endeavour requiring several generations.

The first transgenic fish was produced Zhu et al., (1985) in China, who claimed the transient expression in putative transgenics, although they gave no molecular evidence for the integration of the transgene. The technique has now been successfully applied to a number of fish species. Dramatic growth enhancement has been shown using this technique especially in salmonids. Some studies have revealed enhancement of growth in adult salmon to an average of 3-5 times the size of non-transgenic controls, with some individuals, especially during the first few months of growth, reaching as much as 10-30 times the size of the controls (Hew et al., 1995). The introduction of transgenic technique has simultaneously put more emphasis on the need for production of sterile progeny in order to minimize the risk

of transgenic stocks mixing in the wild populations. The technical development has expanded the possibilities for producing either sterile fish or those whose reproductive activity can be specifically turned on or off using inducible promoters. This would clearly be of considerable value allowing both optimal growth and controlled reproduction of the transgenic stocks while ensuring that any escaped fish would be unable to breed.

An increased resistance of fish to cold temperatures has been another subject of research in fish transgenics for the past several years (Fletcher et al., 2001). Coldwater temperatures pose a considerable stressor to many fish and few are able to survive water temperatures much below 0-1 °C. This is often a major problem in aquaculture in cold climates. Interestingly, some marine teleosts have high levels (10-25 mg/ml) of serum antifreeze proteins (AFP) or glycoproteins (AFGP) which effectively reduce the freezing temperature by preventing ice-crystal growth. The isolation, characterization and regulation of these antifreeze proteins particularly of the winter flounder, *Pleuronectes americanus* has been the subject of research for a considerable period in Canada. Consequently, the gene encoding the liver AFP from winter flounder was successfully introduced into the genome of Atlantic salmon where it became integrated into the germ line and then passed onto the off-spring (F3) where it was expressed specifically in the liver (Hew et al., 1995). The introduction of AFPs to gold fish also increased their cold tolerance, to temperatures at which all the control fish died (12 h at 0° C; Wang et al., 1995). Similarly, injection or oral administration of AFP to juvenile milkfish or tilapia led to an increase in resistance to a 26 to 13° C drop in temperature. The development of stocks harbouring this gene would be a major benefit in commercial aquaculture in counties where winter temperatures often border the physiological limits of these species.

The most promising tool for the future of transgenic fish production is undoubtedly in the development of the embryonic stem cell (ESC) technology. These cells are undifferentiated and remain totipotent so they can be manipulated in vitro and subsequently reintroduce into early embryos where they can contribute to the germ line of the host. This would facilitate the genes to be stably introduced or deleted (Melamed et al., 2002). Although significant progress has been made in

several laboratories around the world, there are numerous problems to be resolved before the successful commercialization of the transgenic brood stock for aquaculture. To realize the full potential of the transgenic fish technology in aquaculture, several important scientific breakthroughs are required. There include:

1. More efficient technologies for mass gene transfer
2. Targeted gene transfer technologies such as embryonic stem cell gene transfer
3. Suitable promoters to direct the expression of transgenes at optimal levels during the desired developmental stages
4. Identified genes of desirable traits for aquaculture and other applications
5. Information on the physiological, nutritional, immunological and environmental factors that maximize the performance of the transgenics of the transgenics and
6. Safety and environmental impacts of transgenic fish.

Work on producing aquatic genetically modified organisms (GMO) of relevance for the ornamental trade has, so far, been carried out (in particular) in Asia. In Singapore, for example, transgenic Zebra Fishes (*Daniorerio*) that glow in green or red under UV-light were first produced some years ago. And, in several countries, research continues to find new areas for employing genetic engineering in the production of new fish varieties. Efforts do not only focus on improving colour and shape, but also on developing characteristics such as faster growth, resistance to infection and tolerance of lower temperatures. This last point, in particular, would certainly open up the debate on invasiveness and environmental risks.

Chromosome Engineering

Chromosome sex manipulation techniques to induce polyploidy (triploidy and tetraploidy) and uniparental chromosome inheritance (gynogenesis and androgenesis) have been applied extensively in cultured fish species (Pandian and Koteeswaran, 1998; Lakra and Das, 1998). These techniques are important in the improvement of fish breeding as they provide a rapid approach for gonadal sterilization, sex control, improvement of hybrid viability and cloning. Most vertebrates are

diploid meaning that they possess two complete chromosome sets in their somatic cells. Polyploidy individuals possess one or more additional chromosome sets, bringing the total to three in triploids, four in tetraploids and so on. Induced triploidy is widely accepted as the most effective method for producing sterile fish for aquaculture and fisheries management. The methods used to induce triploids and other types of chromosome set manipulations in fishes and the applications of these biotechnologies to aquaculture and fisheries management are well described (Purdom, 1983; Chourrout, 1987; Thorgaard, 1983; Pandian and Koteeswan, 1998). Tetraploid breeding lines are of potential benefit to aquaculture, by providing a convenient way to produce large numbers of sterile triploid fish through simple interploidy crosses between tetraploids and diploids (Chourrout et al., 1986; Guo et al., 1996). Although tetraploidy has been induced in many finfish species, the viability of tetraploids was low in most instances (Rothbard et al., 1997).

In teleosts, techniques for inducing sterility include exogenous hormone treatment (Hunter and Donaldson, 1983) and triploidy induction (Thorgaard, 1983). The use of hormone treatments, however could be limited by governmental regulation and a lack of consumer acceptance of hormone treated fish products. Triploidy can be induced by exposing eggs to physical or chemical treatment shortly after fertilization to inhibit extrusion of the second polar body (For reviews see Purdom, 1983; Thorgaard, 1983 and Ihssen et al., 1990) triploid fish are expected to be sterile because of the failure of homologous chromosomes to synapse correctly during the first meiotic division. Methods of triploidy induction include exposing fertilized eggs to temperature shock (hot or cold), hydrostatic pressure shock or chemicals such as ancolchicines, cytochalasin-B or nitrous oxide. Triploid can also be produced by crossing tetraploids and diploids. Tetraploid induction involves fertilizing eggs with normal sperm and exposing the diploid zygote for physical or chemical treatment to suppress the first mitotic division.

Gynogenesis is the process of animal development with exclusive maternal inheritance. The production of gynogenetic individuals is of particular interest to fish breeders because a high level of inbreeding can be induced in single generation. Gynogenesis may also be used to produce

all-female populations in species with female homogamety and to reveal the sex determination mechanisms in fish. It is convenient to use all female gynogenetic progenies (instead of normal bisexual progenies) for sex inversion experiments. Methodologies combining use of induced gynogenesis with hormonal sex inversion have been developed for several aquaculture species (Gomelsky et al., 2000). Androgenesis is the process by which would have commercial application in aquaculture. It can also be used in generating homozygous lines of fish and in the recovery of lost genotypes from the cryopreserved sperms. Androgenetic individuals have been produced in a few species of cyprinids, cichlids and salmonids (Bongers et al., 1994).

Cryopreservation of gametes or gene banking

Cryopreservation is a technique, which involve long-term preservation and storage of biological material at a very low temperature usually at -196°C , the temperature of liquid nitrogen. It is based on the principle that very low temperature tranquilizes or immobilizes the physiological and biochemical activities of cell, thereby making it possible to keep them viable for very long period. The technology of cryopreservation of fish spermatozoa (milt) has been adopted for animal husbandry. The first success in preserving fish sperm at low temperature was reported by Blaxter (1953) who fertilizes Herring (*Clupea harengus*) eggs with frozen thawed semen. The spermatozoa of almost all cultivable fish species has now been cryopreserved (Lakra, 1993). Cryopreservation overcomes the problem of male maturing before female, allow selective breeding and stock improvement and enables the conservation (Harvey, 1996). Most of the plant varieties that have been produced are based on the gene bank collections. Aquatic gene bank however suffers from the fact that at present it is possible to cryopreserve only the male gametes of finfishes and there is no viable technique for finfish eggs and embryos. However, the report on the freezing of shrimp embryos by Subramoniam and Newton (1993) and Diwan and Kandaswami (1997) look promising. Therefore, it is essential that gene banking of cultivated and cultivable aquatic species be undertaken expeditiously.

Marker assisted genetic improvement

It is difficult to develop a sustainable enhanced production based on wild broodstock. Therefore,

it is of great importance to develop a system independent of wild broodstock by controlled reproduction involving broodstock development, breeding and seed production. The development of selective breeding programme is regarded as an important tool in order to domesticate marine finfishes and improve important economic traits. The information on genetic variation is essential for conservation and stock improvement programmes. In farmed animals and plants, it has been demonstrated that systematic selection is an efficient way of improving production traits and thereby produce fast growing animals with high quality.

Selective breeding programs in aquaculture make use of family information, which requires that families are kept separately until the fish are large enough to be physically tagged. This imposes major economic and practical problems, and can induce environmental effects common to full-sibs. Identification of family groups by use of DNA markers has the potential to overcome these problems. Using this technology, fish from different families could be reared together in the same tank even from the egg stage. As the need to keep each family in a separate tank is circumvented, using DNA markers would allow larger number of families to be tested, without increasing the number of tanks, and thus facilitating the use of higher selection intensities without rapid accumulation of inbreeding.

Due to their high polymorphism, microsatellite markers are useful for genetic tagging. Microsatellites have successfully been used to empirically reconstruct pedigrees in fish populations with families mixed from hatching. In an experiment with Atlantic salmon families, using four highly polymorphic microsatellites was sufficient to assign at least 99% of the offspring to the correct pair with 100 crosses involving 100 males and 100 females. An additional polymorphic microsatellite was required for correctly assigning 99% of the offspring when the 100 crosses were produced with 10 males and 10 females. This study demonstrated that parental assignment is feasible with the DNA markers currently available in several fish species.

Both the efficiency and costs of microsatellite based pedigree analysis should be considered before this method is included in a breeding program. Practically, the parents and the mixed

offspring are genotyped by PCR amplifying the appropriate microsatellite loci from crude DNA extracts from small non-destructively sampled quantities of tissue such as fish scales, mucus, and fin clip. Using this protocol, approximately 2000 fish from a mixture of 500 families can be screened for 10 markers in less than a month allowing 99 % of the fish to be parental assigned. Allocation of offspring to families without parental genotypes is also possible, provided enough fish are sampled to obtain sufficient representation of all families, but a larger number of markers are required than for parentage analysis, with probably more than one hundred microsatellites required.

The steady decreasing costs of genetic tagging may still not compete with traditional physical tagging. As the genotype information is detached from the individual, genetic tagging implies that the fish has to be retyped each time its performance is evaluated or individuals are selected. Alternately, fish can be physically tagged for re-identification following genotyping and parentage assignment. Thus the cost of implementing DNA markers in selection programs could be considerable. However it should soon be possible to apply recent developments in human genetics such as DNA chips, where many SNP markers can be genotyped simultaneously and cheaply, in the aquaculture species, and this could dramatically reduce the cost of genetic tagging. DNA markers have several other applications within fish management as well, including evaluation of inbreeding levels, stock identification, movement of released or escaped fish and their possible genetic interactions with wild stocks.

Biotechnology and fish health management

Disease problem area is the major constraint for development of aquaculture. Biotechnological tools such as molecular diagnostic methods, use of vaccines and immunostimulants are gaining popularity for improving the disease resistance in fish and shellfish species. For viral diseases, avoidance of the pathogen is very important. In this context there is a need to develop rapid method for detection of the pathogen. Biotechnological tools such as gene probes and polymerase chain reaction (PCR) are showing great potential in this area. Gene probes and PCR based diagnostic methods have developed for a number of pathogens affecting fish and shrimp (Karunasagar, 1999). In case of

finfish aquaculture, numbers of vaccines against bacteria and viruses have been developed. Some of these have been conventional vaccines consisting of killed microorganism but new generation of vaccines consisting of protein subunit vaccine, genetically engineered organism and DNA vaccine are currently under development.

In the vertebrate system, immunization against disease is a common strategy. However the immune system of shrimp is rather poorly developed, biotechnological tools are helpful for development of molecule, which can stimulate this immune system of shrimp. Recent studies have shown that the non specific defence system can be stimulated using, microbial product such as lipopolysaccharides (LPS), peptidoglycans or glucans (Itami et al., 1998). Among the immune-stimulants known to be effective in fish, glucan and levamisole enhance phagocytic activities and specific antibody responses (Sakai, 1999).

In the attempts to combat viral and bacterial pathogens threatening commercial stocks, the utilization of DNA vaccines has been promising. This technique is based on the injection of DNA encoding part of the antigen, usually a bacterial outer membrane or viral capsid protein, in the fish muscle. Here the protein will be synthesized, and the production of antibodies against the foreign protein is induced. A significant degree of protection against infectious hematopoietic necrosis virus (IHNV) was found in Atlantic salmon after vaccination with a gene construct containing an IHNV glycoprotein. Similarly, protection against viral haemorrhagic septicaemia virus (VHS) was induced in vaccinated rainbow trout. The main disadvantage of these approaches is they require detailed information about the structure, conformation, and the encoding sequence of the pathogen's protein. An alternative approach to increase the resistance of fish to pathogens is to target the nonspecific immune response through use of antimicrobial peptides, which are found in both vertebrates and invertebrates. Short peptides consisting of 30-50 amino acids with strong antimicrobial activity have been isolated from the skin mucus of several fish species. Recently, manipulation of antimicrobial cecropin genes in the Japanese medaka and channel catfish produced strains of transgenic fish resistant to infection by fish bacterial pathogens.

Future Prospects

The completion of the Human Genome Project inspired the entire world and triggered the start of a genomics revolution. Accompanying this revolution was a complete change in the way science was conducted in the field of life sciences. Without exception, the waves produced by the genome revolution are now having a tremendous impact on aquaculture genomics and aquaculture genetics in general. This raises new challenges for aquaculture geneticists, breeders, and fisheries managers regarding how to best use the huge amount of genomic information now available, and how to master and apply continuously changing genome technologies to aquaculture and fisheries.

The applications of molecular techniques in aquaculture are promising, but still somewhat uncertain. While high costs seem to be the only hindrance for widespread application of DNA markers for identification purposes and marker assisted selection, the situation regarding commercial use of genetically modified fish is more complex. Although the potential importance of gene transfer technology is large, a major concern relates to the possible impact, which release or accidental escapes of gene-modified individuals may have on natural ecosystems. Other controversial aspects are related to animal welfare, food safety and the public perception of gene manipulation in general. To which extent such issues will constrain the future use transgenic animals in applied aquaculture production remains to be seen.

Other technologies are also rapidly emerging which are either being used or are likely to be used in the future in the aquaculture species. For example micro-array technology, where an array of DNA or protein samples are hybridized with probes to study gene expression of a large number of genes simultaneously, can be used to determine which genes are involved in response to disease, expression of meat colour and other important issues. Already, a microarray containing DNA probes for 3700 DNA sequences or genes is available in salmon, and will be used to compare gene expression in disease challenged and non-challenged fish (Davidson and Koop, 2003). Such technology has the potential to contribute very large amounts of information on the genes and pathways of genes, which affect the economic traits in aquaculture species.

Biotechnological research and development are growing at a very fast rate. The biotechnology has assumed greatest importance in recent years in the development of fisheries, agriculture and human health. The science of biotechnology has endowed us with new tools and tremendous power to create novel genes and genotypes of plants, animals and fish. The application of biotechnology in the fisheries sector is a relatively recent practice. Nevertheless, it is a promising area to enhance fish production. The increased application of biotechnological tools can certainly revolutionise our fish farming besides its role in biodiversity conservation. The paper briefly reports the current progress and thrust areas in the transgenesis, chromosome engineering, use of synthetic hormones in fish breeding, biotechnology in health management and gene banking.

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Health management in hatchery and grow-out mariculture

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Introduction

Mariculture has been steadily growing over the last few decades. To satisfy the increasing demand of local and export markets for fish and to control overexploitation of marine fish species, many countries are expanding mariculture activities. Disease is one of the most limiting factors in mariculture. Intensification of mariculture favours pathogens, which increase disease outbreaks. Diseases are broadly classified into infectious and non-infectious diseases. Infectious diseases are further divided into four groups based on the nature of the pathogen: viral, bacterial, parasitic, or fungal. Non-infectious diseases are divided into neoplastic diseases, genetic and environmentally induced diseases, and nutritional deficiency diseases. Sustainable aquaculture production can only occur when fish are healthy and free from disease. Fish disease management is a combination of preventing the onset of disease and measures to reduce losses from disease when it occurs. Fish cultured in floating cages become particularly susceptible to disease when various environmental parameters such as temperature, salinity, dissolved oxygen and suspended particles fluctuate suddenly or widely, or following rough, although often unavoidable, handling operation. Once conditions suitable for pathological changes develop, progress to disease in the warm water environment is rapid. Early detection of behavioural changes and clinical signs in the cultured animals are critical for proper diagnosis of the disease.

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

Fish health management

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

Predisposing factors

- Fish stocks living under stressful conditions are less able to defend against a pathogen and hence will become sick more readily. Fish that are well cared for generally do not become sick even in the presence of a pathogen. The most common error in fish husbandry is overstocking. This leads to problems such as:
- Fish to fish aggression
- Increased fish and feed wastes
- Ease of disease spread,
- Increased concentration of pathogens
- Resultant poor water quality

High fish density, stress, and ease of transmission increase susceptibility of the fish population

to diseases and parasites. In marine aquaculture, diseases present in wild fish can infect cultured fish and spread rapidly.

Types of fish diseases

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

- Infectious diseases. Infectious diseases are broadly categorized as parasitic, bacterial, viral, or fungal diseases.

Common Diseases of Cobia (*Rachycentron canadum*)

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

Common Diseases of Pompano (*Trachinotus blochii*)

S.No	Disease	Causative agent
1	White spot disease	Ciliate protozoan, <i>Cryptocaryon irritans</i>
2	Cardiac myxosporidiosis	Myxosporidian protozoan, <i>Henneguya</i> sp.

3	Monogenetic trematode infestation	<i>Bicotylophora trachinoti</i> - gills <i>Benedenia</i> sp-body
4	Fatty degeneration	Dietary deficiency- protein
5	Parasitic dermatitis (infestation)	Sea lice (<i>Calligus elongatus</i>)
6	Amyloodiniosis	<i>Amyloodinium ocellatum</i>

Common diseases of marine ornamental fishes

S.No	Disease	Causative agent
1	Red pest	Gram negative bacteria
2	Fin Rot	Gram negative bacteria
3	Fish tuberculosis	<i>Mycobacterium</i> sps
4	External Gas Bubble disease	Various causes Commonly caused by excess gas in the system, brought about by super-saturation of gas in high pressure water mains

- Vibriosis is a bacterial disease causing significant losses of fish in marine fish farms. Cobia, Grouper, seabream, snapper and pompano species are affected. Vibriosis results in severe skin, muscle, fin, eye and internal organ damage of fish. Diagnosis of the disease requires bacteriological culture of kidney, spleen, skin or eye lesions.
- Non-infectious diseases: Non-infectious diseases can be broadly categorized as environmental, nutritional, or genetic.
- A hygienic fish culture environment is essential to the health and productivity of farming operations. The reasons for this include:
- Disease risks are increased in poor and polluted environments.
- Quality of the product depends on clean and healthy environments.

The culture environment incorporates the following components

- Physical farm infrastructure e.g. fish cages, floats, nets, and utensils.
- Water quality e.g. dissolved oxygen and microbial contamination.
- Seabed sediments e.g. solid wastes measured as carbon, nitrogen and phosphorus.
- Introduced chemicals e.g. antibiotics, metals and pesticides.
- Successfully curing the fish and eliminating the disease or cause of distress.

1) Cobia fingerlings affected with Vibriosis



Fig. 1. Eye: Bilateral exophthalmus

Husbandry practices:

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

Preventive measures

- Preventing the introduction of pathogens by proper quarantine procedures
- Maintenance of good water quality
- Avoidance or reduction of environmental stressors
- Adequate nutrition
- Isolation of cultured animals from feral stocks
- Regular immunization against major pathogens

Steps to solve a disease problem

- Determining that a problem exists.
- Identifying the cause of the disease or source of the distress



Fig.2. Stomach: Haemorrhagic gastritis

II) Histopathology

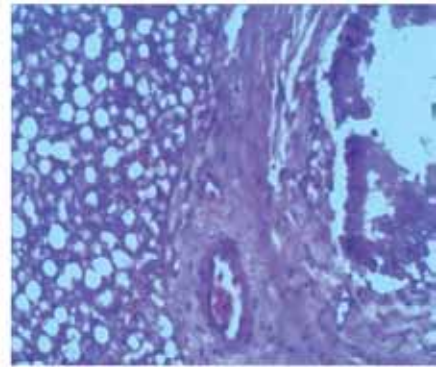
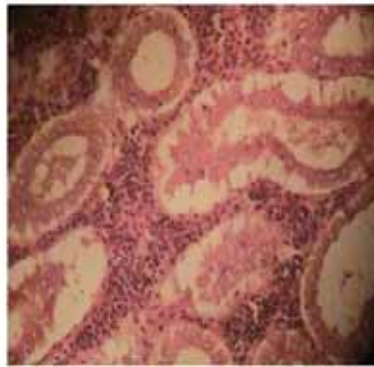
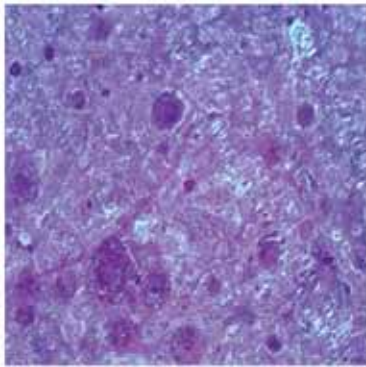


Fig.3 Spleen: Liquefactive necrosis H&E

Fig.4. Kidney : Acute tubular

Fig.5. Liver: Fatty degeneration-H&E

III) Genetic anomalies



Fig.6. Undeveloped upper maxilla



Fig. 7. Scoliosis- vertebral anomalies



IV) Pompano affected with sea lice (*Caligus elongatus*) infestation



Fig.8. *Caligus elongatus*

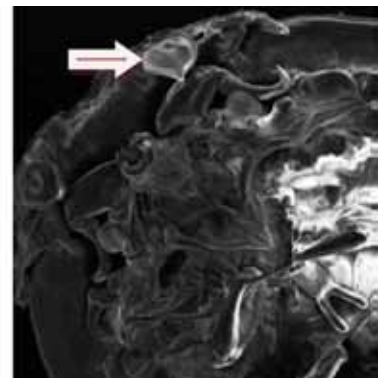


Fig.9. SEM view presence of lunules (arrow)

V) Pompano parasitic infestation due to *Amyloodinium. Ocellatum*



Fig.10. *A. ocellatum*:Gill- Adult

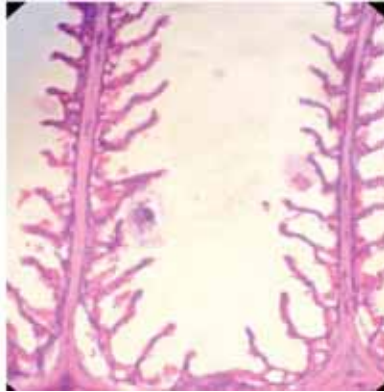


Fig.11.Gill: Hypertrophy of the secondary lamellae H&E feeding stage-trophont

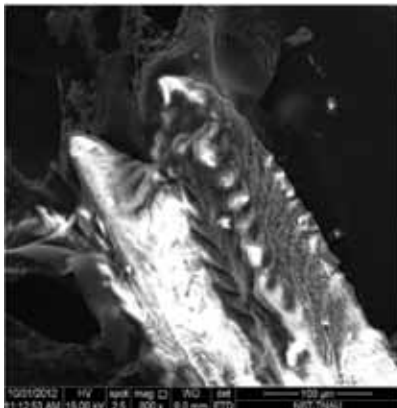
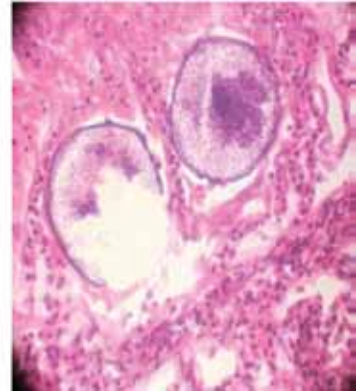
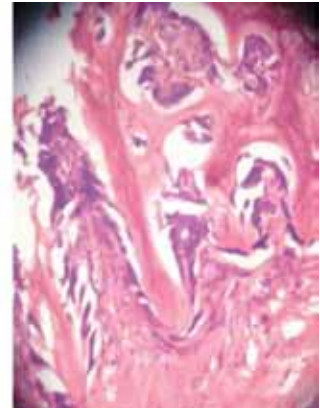


Fig.12. SEM Gill: Hypertrophy lary cyst adenoma H&E



Fig.13.Pompano: Tail tumour Tail tumour :Papil



Sea cage farming of cobia

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Introduction

In recent years interest in aquaculture is gaining greater momentum. The breeding and rearing of aquatic plants and animals in enclosures/confinements, has increased mainly due to over exploitation of aquatic resources and declining of fish catches in major traditional fishing grounds at a global level. The farming of fish is widely recognized as the best alternative to meet the protein requirements of the expanding population and to provide them with alternative livelihood opportunities for their socio-economic upliftment. In many countries, especially in the developing world, fish and other aquaculture products serve as the main source of cheap protein to combat malnutrition and under-nutrition. Fish are having essential amino acids that are often lacking in cereal protein substitutes. Value-wise, cultured fish products compete with poultry and livestock in the local market. Nutrition-wise, however, aquaculture products are more efficient in converting food into body tissue than poultry or livestock. Aquaculture is the fastest growing animal food producing sector in the world. It has delivered growth in production volumes of almost 8% per annum over the past 50 years, approximately twice the rate of global GDP growth during this period. From output of less 1 million tonne per year in the 1950's, aquaculture now produces almost 100 million tonnes per year. Globally, fish today provides more than 1.5 billion people with almost 20% of their average per capita intake of animal protein, and 3 billion people with 15% of such protein. The average person consumes 18 kilograms of fish products per year. With a growing global population and health awareness the per capita consumption of fish and fisheries products are expected to grow further. Commercially

important marine fishes can be cultured in any of the four culture systems like ponds, raceways, recirculation systems or cages. In the simplest term, a cage is an enclosure in the water body whereby the juveniles of aquatic animals are kept, fed and grown to a marketable size. Cage culture uses existing water resources (ponds, rivers, estuaries, open ocean, etc.) but confines the fish inside some type of mesh enclosure. The mesh retains the fish, making it easier to feed, observe and harvest them. The mesh also allows the water to pass freely between the fish and surrounding water resource, thus maintaining good water quality by removing wastes. In recent years, cage culture has emerged as the most viable method of sea farming.

Cage culture probably originated with fishermen who used cages to accumulate fish for market. Over time, they learned to feed the fish in these cages to increase their size and improve their overall health. The first cages used for just holding fish were probably developed in Southeast Asia at the end of the 18th century. These cages were constructed of wood or bamboo and the confined fish were fed trash fish and food scraps. Modern cage culture in the U.S. began in the 1950s with the advent of synthetic materials suitable for cage construction. There has been little research on marine cage systems because of regulatory issues, a limited number of good quality sites and high cost of research. In freshwater sector, cage culture allows farmers to use existing water resources that may or may not be used for other purposes. The fish produced are usually sold to local niche markets. As wild-capture fisheries have declined and aquaculture has expanded, these niche markets have also grown. As a result to cater the demand more entrepreneurial opportunities have grown

for cage farming. The cage culture was initiated in Norway during 70s and developed into an organised industry, particularly for salmon farming. Similarly the cage culture has spread in South East Asian countries for culture of a variety of fishes. The major advantage in these countries is that they have large, calm and protected bays to accommodate the cages safely against natural bad weather conditions.

Advantages of Cage Culture

1. Effective use of Resources

Cage culture can be established in any suitable body of water, including open seas, backwaters, lagoons or river mouths with proper water quality, seed, feeding strategies, access and permission from local authorities. This flexibility makes it possible to exploit underused water resources to produce fish.

2. Low investment

The investment for pond construction and its associated infrastructure (electricity, roads, water wells, etc.) are much higher than the cage farming, which is practiced in an existing water body and can be less expensive. At low densities (when compared to pond water spread area) cages placed in open seas, backwater and lagoons do not require aeration. Cage materials are not much expensive and can be mended with little experience.

3. Simple farming operations

In cage farming, observation of the growth and health status of the fish is easy and simple. The observation of fish behaviour, especially feeding behaviour, is critical in avoiding problems related to stress and disease outbreak.

4. Easy harvesting methods

Cages are usually harvested by moving them into shallow water, crowding the fish into a corner of the net. Otherwise, the cage net can be lifted partially out of the water so that the fish are crowded into a smaller volume, and then it can be harvested. This makes it possible to partially harvest fish from cages as and when needed for local markets.

5. Multi-use of water resources

The confinement of fish in cages will not affect other uses of the water resource, such as fish-

ing, boat-~~ing~~, swimming, irrigation or live-stock watering.

Cage farming requires low capital investment and the farmer can expand production with additional cages or intensify production by increasing the stocking density at an optimal level.

Species selection

Cage culture in open seas requires a fish variety with the basic characters like, suitability for marketing, commercial importance, consumer accepted fish, easy to culture, adaptability to the cage environment, acceptance to artificial diets, faster growth rate and resistant to common diseases. A variety of commercially important marine fishes including, Cobia (*Rachycentron canadum*), Seabass (*Lateolabrax niloticus*), Snappers (*Lutjanus* sp.), Carangids (*Trachinotus* sp.) and Groupers (*Epinephelus* sp.) and lobsters are highly suitable for cage farming. Commercial level seed production technology for majority of these fishes has been developed in many of the South East Asian countries.

Cobia (*Rachycentron canadum*)

Cobia has gained popularity as a good candidate for mariculture due to its rapid growth and white meat of versatile use. It is considered as one of the most promising candidates for warm-water marine fish aquaculture in the world. Being the only member of the family Rachycentridae, it is found in the warm, temperate to tropical waters of the West and East Atlantic, throughout the Caribbean and in the Indo-Pacific off India, Australia and Japan. To date, research and development of cobia aquaculture has been initiated in over 23 countries and territories, half of them in the Asian-Pacific region. Global aquaculture production of cobia has been increased rapidly from only 9 tonnes in 1997 to nearly 30,000 tonnes in 2007. Statistics of FAO (2009) show that since late 1990, cobia aquaculture production has been steadily expanding in Asia, primarily in Taiwan, Vietnam and China, but also in other Southeast and Indo-Pacific Asian countries including the Philippines, Indonesia, Iran and Reunion Island. Although cobia production is expanding rapidly, combined production of Asian countries is still rather lower.

Cobia farming techniques developed by CMFRI

India is late starter in cobia research and the seed production of cobia was achieved for first time in India by the Mandapam Regional Centre of Central Marine Fisheries Research Institute (CMFRI). Later the farming protocols in the High Density Polyethylene (HDPE) cages and Galvanized Iron (GI) cages with different feeding strategies were developed, tested and validated. Out of this farming trials an economically viable farming methods has been evolved. These farming methods have been executed in a participatory farming demonstration with M/s. Vitality Aquaculture Pvt. Ltd., Tuticorin and successful harvest of cobia was made during May 2013 in the presence of the Director General, ICAR, New Delhi. The basic protocols followed for cage culture of cobia in different phases are narrated as below:-

Nursery Phase 1

The 4 weeks old fingerlings were reared for 6 weeks indoor (Nursery Phase 1) followed by 8 weeks outdoor (Nursery Phase 2) before stocking in grow-out cages. The nursery phase 1 can be carried out in FRP tanks of 7 ton capacity with 5 ton filtered sea water. The stocking density has to be kept as 8 nos. per litre. The fingerlings have to be fed with INVE (Thailand) formulated diet (assorted size from 400 μ to 1200 μ) thrice daily. The weaning to chopped low-value fishes can be practised during the last week of this phase. The water exchange has to be done 100% daily.

Nursery Phase 2

The nursery phase 2 has to be carried out in specially designed sea cages. These nursery cages should be made of HDPE pipes or GI Pipe (C - Class type) material. The dimension of the square sea cage has to be kept as 4x4 meter with the handrail fixed at one meter height from the base otherwise a circular cage of 6 meter dia can be used. The net cages fabricated with HDPE ropes of 2.5 mm thickness and the mesh size has to be used are 20 mm for inner net cage and 40 mm for outer net cage. The depth of the net cage shall be kept 3 meters from the base. The shape of the net cages has to be maintained with ballast. The buoyancy of the cages can be enabled by tying HDPE drums with the cage frame and has to be moored with two numbers of Galvanized Iron (GI) anchors of 70/100 kg each in opposite directions.

The fingerlings from nursery phase 1 have to be transferred to these floating nursery sea cages. The stocking density biomass at this phase can be maintained at 1.8-3.0 kg/m³. The fingerlings have to be fed @ 5% total biomass of fish with chopped low-value fishes (Sardine, lesser sardine, rainbow sardine, etc.) twice daily. Net cages have to be changed based on the subjective assessment of clogging of the net in order to have sufficient water exchange. Random sampling has to be carried out weekly with the sample size of 30 nos. per cage. This phase can be continued for about 4 weeks.

Grow-out phase

The grow-out culture has to be carried out in circular floating sea cages of 6 meter diameter. The cage frames should be made up of HDPE pipes or GI pipes. The handrail has to be fixed at half meter height from the base. The space between inner and outer rings of the cage has to be kept as one meter. The net cages fabricated with HDPE ropes of 2.5 mm thickness and the mesh size of 40 mm for inner net cage and 60 mm for outer net cage has to be used. The depth of the net cages should be maintained at 4.0 meters from the base. The shape of the net cages can be maintained with circular ballast. The cages were floated and moored as mentioned in Nursery Phase 2. The juveniles from nursery phase 2 have to be transferred to these grow-out sea cages. The stocking density at this phase has to be maintained at 3.0-5.0 kg/m³ or 750 nos of juvenile cobia per cage. The juveniles can be fed @ 5% total biomass of fish with chopped low-value fishes (sardine, lesser sardine, rainbow sardine, etc.) once daily. Net cages have to be changed based on the subjective assessment of fouling of the net in order to have sufficient water exchange. Random sampling has to be carried out at monthly intervals with the sample size of 30 nos. per cage. The entire grow-out culture can be carried out for a period of 6- 7 months.

Performance

The fingerlings stocked in indoor nursery at around 2 grams and will attain an average weight of 45 grams in 6 weeks, followed and about 70 grams in another 4 weeks of outdoor nursery rearing. The juveniles would reach an average weight of 1.0 kg in 4 months and 2.5 – 3.0 kg in 6- 7 months of grow-out culture in sea cages. The

grow-out fishes would reach an average weight of 7.0 kg with a maximum weight of 8.0 kg within the culture period of one year which is almost 100 times the growth of the initial weight.

The unit cost estimate, performance of production and economics of operation gained through the farming trials and participatory demonstration were worked out and given below:-

Unit cost economics for cage farming of cobia (in a 6 m diameter GI cage)

Sl. No	Head of expense	Cost in Rs.
Capital Expenditure		
Cage and Net		
1	Cage (6 meter dia) made of 'C' class GI Pipe of 1.5 inch dia)	50,000.00
2	Mooring	15,000.00
3	Nets (2 Inner net and one outer net with ballast pipe)	60,000.00
	Sub Total	1,25,000.00
Operational Expenditure*		
1	Cost of 750 Numbers of cobia seeds @ Rs 10/seed	7,500.00
2	Transportation	5,000.00
3	Cost of 12.82 tonnes of low value fishes @ Rs.25,000/tonne	3,20,500.00
4	Labour Charges @ Rs.1000/ Person for 7 months for 2 persons	14,000.00
5	Boat Hire & Fuel Charges	10,000.00
6	Harvesting Charges	5,000.00
7	Miscellaneous Expenses	10,000.00
	Sub Total	3,72,000.00
	Grand Total of Capital & Operational expenditure	4,97,000.00

*Item No. 4 &5 worked out based on the average expenditure/month for a cluster of 10 cages

Sl. No	Production Estimates	
1	Survival 95% = 712 fishes	
2	Feed Conversion Ratio = 1 : 6	
3	Average size of each fish at the time of harvest = 3kg	
4	Total harvest = 2.136 tonnes/cage	
5	Sale price of the produce @ Rs.280/kg = Rs. 5,98,080/-	
	Gross Income from the harvest = Rs. 5,98,080/-	
Economics		
1	Gross income from Harves	- Rs. 5,98,080/-
2	Operational expenditure	- Rs. 3,72,000/-
3	Gross income – Operational expenses	- Rs. 2,26,080/-
	Net Profit = Rs. 2,26,080/-	
4	Partial repayment of the capital expenditure (Capital cost Rs. 1,25,000 – Subsidy Rs. 50,000 Repayment of capital @ Rs. 25,000/year x 3 years	- Rs. 25,000/year - Rs. 75,000)
5	Interest in the total project cost @ 11%	- Rs. 52,800/-
6	Part of Capital + interest = Rs. 25,000 + 52,800	- Rs. 77,800/-
7	Rs. 2,26,080 – 77,800 = 1,48,280/-	
	Net profit (after repayment of interest & part of capital expenditure)	Rs. 1,48,280/-

Unit cost economics for a cluster of 10 cages to take up farming of cobia

Sl.No	Head of expense	Cost in Rs.
Capital Expenditure		
Cage and Net		
1	Cost of 10 Cages (6 meter dia) made of 'C' class GI Pipe of 1.5 inch dia)	5,00,000.00
2	Mooring materials for 10 cages	1,50,000.00
3	Nets (2 Inner net and one outer net with ballast pipe) for 10 cages	6,00,000.00
	Sub Total	12,50,000.00
Operational Expenditure*		
1	Cost of 7,500 Numbers of cobia seeds @ Rs 10/seed	75,000.00
2	Transportation	50,000.00
3	Cost of 128.250 tonnes of low value fishes @ Rs.25,000/tonne	32,06,250.00
4	Labour Charges @ Rs.10,000/ Person/month for 2 Persons X 7 months	1,40,000.00
5	Boat Hire & Fuel Charges	1,00,000.00
6	Harvesting Charges	50,000.00
7	Miscellaneous Expenses	1,00,000.00
	Sub Total	37,21,250.00
	Grand Total of Capital & Operational expenditure	49,71,250.00

Sl. No	Production Estimates
1	Survival 95% = 7125 fishes
2	Feed Conversion Ratio = 1 : 6
3	Average size of each fish at the time of harvest = 3kg
4	Total harvest = 21.375 tonnes/cage
5	Sale price of the produce @ Rs.280/kg = Rs. 59,85,000/-
	Gross Income from the harvest = Rs. 59,85,000/-

Sl. No	Economics
1	Gross income from Harvest = Rs. 59,85,000/-
2	Operational expenditure = Rs. 37,21,250/-
3	Gross income – Operational expenses = Rs. 22,63,750/-
	Net Profit = Rs. 22,63,750/-
4	Partial repayment of the capital expenditure = Rs. 25,000/year/cage (Capital cost Rs. 12, 50,000 – Subsidy Rs. 5,00,000 = Rs. 7,50,000) Repayment of capital @ Rs. 2,50,000/year x 3 years
5	Interest in the total project cost @ 11% = Rs. 5,46,838/-
6	Part of Capital + interest = Rs. 2,50,000 + 5,46,838 = Rs. 7,96,838/-
7	Rs. 22,63,750 – 7,96,838 = 14,66,912/-
	Net profit (after repayment of interest & part of capital expenditure) = Rs. 14,66,912/-



HDPE Cage (6 meter Dia)



GI Pipe Cage (6 meter Dia)



Cobia fingerlings (50 days old)



Cobia juveniles (While feeding)



Cobia Juveniles (3 kg size)



Harvested Cobia

Capture based aquaculture of red snapper
Lutjanus argentimaculatus **in cages**

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Introduction

Global production from aquaculture has grown substantially, contributing significant quantities to the world's supply of fish for human consumption. This increasing trend is projected to continue in the forthcoming decades also. It is envisaged that the sector will contribute more effectively to food security, poverty reduction and economic development by producing 83 million tonnes of aquatic food by 2030– with minimum impact on the environment and maximum benefit to the society with an increase of 37.5 million tonnes from the 2004 level (FAO). Aquaculture is a diverse sector using many strategies for fish production. The harvesting of wild individuals, either as broodstock whose eggs will hatch and develop under culture in ponds or cages, or as early life-history stages for on-growing under confined and controlled conditions is one of the strategies. This system of aquaculture production has been termed by the Food and Agriculture Organization (FAO) as capture-based aquaculture (CBA). It is a worldwide aquaculture practice and has specific and peculiar characteristics for culture, depending on areas and species. There is a worldwide distribution of this practice of the CBA, and some species which are cultured include shrimps (*Penaeidae*), milkfish (*Chanoschanos*), eels (*Anguilla* spp.), yellowtails (*Seriolaspp*), tunas (*Thunnus* spp.) and groupers (*Epinephelus* spp.).

These species are caught and farmed using various techniques and systems, depending on different local cultural, economic and ethnical traditions. In some areas this is typically artisanal, rather than industrial in nature. Economic considerations are the key drivers for capture-based aquaculture. The selection of species for culture reflects their acceptability and demand in local or international markets. Market requirements are determined primarily by people's tastes and customs. As capture-based aquaculture potentially generates higher profits than other aquaculture systems, the market demand for the products and species cultured is high and it is likely that efforts

to promote this activity will significantly increase. This development will be capable of causing a number of very important and diverse effects, not all of them beneficial.



Cage aquaculture is practiced in many part of the world and capture based aquaculture in cages is also popular. Recently Central Marine Fisheries Research Institute (CMFRI) has initiated culturing of marine finfishes in cages and it has proven successful in many maritime states. In this the adoption of sustainable capture based aquaculture initiative by the traditional coastal fishers the state of Karnataka is noteworthy. The participatory approach gave exposure to the local fishers on the finfish rearing aspects besides creating awareness on this lucrative farming technique. Encouraged by this success many fishermen group evinced interest in rearing finfish in suitable farming areas near their backyard. One of the species selected for capture based aquaculture was red snapper *Lutjanus argentimaculatus*. Factors such as their popularity as a food fish, high market price have contributed to substantial interest in red snapper aquaculture.

Site selection

Proper site selection for cage marine culture is of paramount importance as it may considerably affect construction costs, operating costs, growth and survival rate of the fish and the period of usefulness of the cages. Although floating cages can

be usually towed away, sometimes it is not economical to do so. The site selection criteria adopted for aquaculture should be followed in the cage culture also. The site selected should have a minimum depth of 2.5 m, it should be free from pollution, with minimum fouling, should have good circulation of water to remove the waste materials falling from the cage etc. It is better to avoid the areas where phytoplankton blooms occur frequently and places where boats are operated. The place selected should have good accessibility.



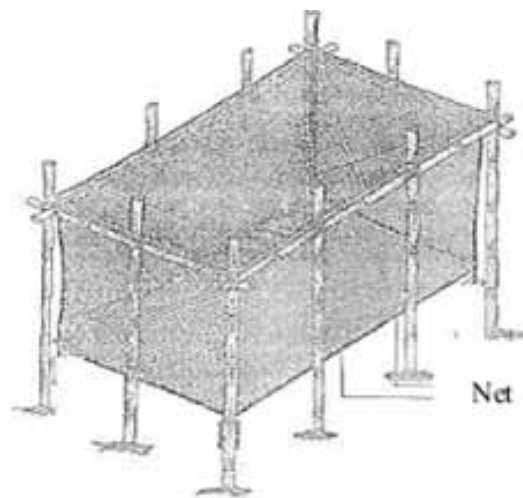
Fish Seed Source

The estuaries are rich source of seed resources of cultivable fishes. In the estuary fishermen use cast nets and dragnets for fishes. Usually small sized fishes thus caught are not of economical value and is discarded. An attempt was made to popularize the concept of capture based aquaculture by judiciously utilizing these seed resources. Thus small sized red snappers which are of low market value were used for the cage culture.



Cage design

The cage size and shape was designed following the conventional cage design (Fig 1) modified to suit the locality. Floating cages of 2.5 m x 2.5 m x 2 m, made of Netlon (mesh of 30 mm) lined with nylon net were fabricated for the fishes. These cages were installed in the estuary. Bamboo poles formed a cage frame to stabilize the





net. The cage was set when the tide recedes and the distance from the cage bottom to the ground was 0.3m and the height from the water surface to the highest point of the cage was 0.75 m. The top was covered with large meshed nets to prevent the escape of the fishes and also the predatory birds. Netlon mesh is used as outer cover to prevent the predation of crabs which is common in the estuaries.

Stocking of fishes

Since it is capture based aquaculture the fingerlings could be stocked continuously as and

when they are caught from the wild. But care should be taken to stock similar sized fishes in one cage as there are chances of cannibalism. For the cages of 2.5 m x 2.5m x 2 m, the stocking density of 500-600 nos. is found to be feasible. The fishes of size range 8-12 cm (15-20 g) were stocked in the cages.

Feeding: Feed used in the cages was trash fish (available in the locality), and the feeding quantity was in line with the body weight. The fish was fed twice per day in the early morning and evening and the feed amount was adjusted in line with the body weight (8% in the first month; 7% in the second month; 6% in the third month and 5% from the fourth month onwards). Feed is sliced into pieces before feeding.



Cage management: Routine cleaning of the outer net has to be done to prevent the clogging of the outer net which would hinder the water circulation inside the cage. Checking of the fishes for diseases and mortality should also be done regularly.

Growth of Red Snapper in cages. The growth of the fish ranged from 90-100 gm. per month with

and the fishes attained about 750-900 g after 8 months of culture. The fishes attained about 1.1kg to 1.3 kg after 13 months of culture.

Harvesting: Partial harvesting of the fishes could be done according to the market demand. About 70% survival is expected in cages. Total production from one cage is estimated at

308kg after 8 months. Approximate price for the fish in local market is Rs.280/

kg (US \$ 4.5) and the amount realized is Rs.86240/- (US\$ 1384).

When the capture based aquaculture is being practiced in high intensity some of the scientific factors has to be taken care. Carrying capacity of the water body where cages are installed is a very important factor. The number of cages should be according to the carrying capacity of the water body and if the number of cages exceeds its carrying capacity, it will effect fish growth and survival. There is a strong need for better data on the biology and fisheries of the species. Accumulation of



uneaten feed and fish excreta under the cage can become an environmental problem, but this can be avoided by selecting a site with good water exchange to install the cage. Capture based aquaculture provides significant positive returns in areas with depressed and marginal economies, and an alternative livelihood for coastal communities. However, the difficulties of marketing fresh fish and supplying markets that demand live fish (e.g. groupers), and the need to expand markets limit its potential. Skill gaps are evident in the sector, including specific knowledge on economics and management, the suitability of individual (new) species for culture, information on their biology and dietary requirements, and marketing. Capture-based aquaculture is labour intensive in its farming and processing operations, and can contribute to poverty alleviation in developing countries.

Legal and security issues: We will have to envisage some difficulties in future development of capture based aquaculture. Security of the cages is the major issue. For leasing the inland waters and estuaries, the provisions are to be made. Leases policies should be guided by a set of rules and principles relevant to public trust responsibilities and should specify the size of farm, duration of farming and other terms of lease. Rents thus collected should be used for development of coastal areas.

Food safety issues: The success of cage culture depends on maintaining good water quality around the fish cages and so it is in the farmer's best interests to minimize environmental impacts. Size and intensity of the process should fit to the size of the water body and water exchange rate. It may facilitate to overcome adverse impacts on water and sediment quality. In common with other types of aquaculture, careful choice of aqua-feed ingredients and on-growing sites, in addition to good management practices, are necessary to avoid the accumulation of chemical and antibiotic residues, in order to ensure the continued safety of farmed products. Capture-based aquaculture provides other opportunities to reduce the risks associated with food safety.

Culture of Silver Pompano *Trachinotus blochii* in coastal aquaculture ponds

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Introduction

Among the many high value marine tropical finfish that could be farmed in India, the silver pompano, *Trachinotus blochii* is one of the topmost, mainly due to its fast growth rate, good meat quality and high market demand. The silver pompano is caught only sporadically in the commercial fishery and hence its availability is rather scarce. It is a much sought after species and hence the demand can only be met through aquaculture. The aquaculture of pompano has been successfully established in many Asia-Pacific countries like Taiwan and Indonesia. The farming can be successfully carried out in ponds, tanks and floating sea cages. The species is pelagic, very active and is able to acclimatize and grow well even at a lower salinity of about 10 ppt and hence is suitable for farming in the vast low saline waters of our country besides its potential for sea cage farming. The shape, colouration and meat quality of this fish is comparable with silver pomfret. In the international market, the dockside price of Florida pompano averaged to \$ 8 /kg and in India, the current price of silver pompano is about Rs.200/kg at the fish landing centres and around Rs.250/kg in the retail markets.

The Central Marine Fisheries Research Institute has initiated aquaculture research on pompano from 2008 and the first successful broodstock development, induced breeding and larval production was achieved in 2011. Following the successful seed production of Silver Pompano, demonstration of farming in brackishwater ponds was initiated by the CMFRI to popularize among the farmers about its suitability for aquaculture. The first farming demonstration from the hatchery produced seed was carried out in a coastal aquaculture pond at Anthervedi Village, East Godavari District, Andhra Pradesh. It has been proven that Silver pompano can be cultured in the brackish water shrimp culture ponds as an alternative species with high survival rate, appreciable FCR and meat quality. These fishes have attained an average weight of 450 grams in 240 days (8 months).

Based on the experience gained on the brackish-water farming of silver pompano, the practices to be adopted for pompano farming are presented.

Pond preparation

The pond has to be dried properly until the cracks appear on the surface. The top layer of the soil containing waste accumulated through previous crop of fish or shrimp has to be removed. Ploughing has to be done to tilt the soil below 30 cm. Feeding areas, corners and side ditches in the pond has to be properly tiled and dried to avoid formation of black soil. The average water pH of 7.5-8.5 would be ideal for pompano farming. The level of lime application during pond preparation depends on the pH of the soil. Hence, the dosage has to be calculated accordingly. Water filling has to be initiated by covering the inlet pipe by using 2 layers of fine nets (100 micron) to avoid introducing other fishes and predators. A week before stocking, the pond must be fertilized with either organic or inorganic fertilizers to stimulate the plankton bloom.

Salinity

Pompano can tolerate wide range of salinities from 5- 40 ppt. However, ideal salinity for farming would be between 15 – 25 ppt and the pond has to be filled with a minimum water level of 100 cm prior to stocking of fish seeds. During the entire culture period 1.5 meter water depth has to be maintained.

Nursery Rearing and Seed Stocking

Hatchery produced pompano fingerlings of 1 inch size can be stocked in happas/ pens of 2 meter length, 2.0 meter width and 1.5 meter depth. In each happa about 200 fingerlings can be stocked. While stocking care should be taken to avoid agitation of the pond bottom and too many persons getting into the pond may increase the suspended solid load in the water, which may cause gill chocking of the fish fingerlings leading to mortality. Initially the fishes have to be reared in happas for 60 days or until they attain 10 – 15



Adult Pompano



Pompano Fingerlings (1.5 inch size)



First Supply of Pompano to a farmer



Nursery rearing in hapa



Pond Culture of pompano



Feeding with Extruded Floating Pellet feed



Pompano (25 Grams size)



Pompano grown to 200 grams size



Sampling of Pompano



Harvested Pompano

grams size and thereafter it can be released into the pond. The mesh size of the happa could be initially at 4 mm size and it can be changed with 8mm mesh size happas after 30 days. The stocking density in happa could be maintained as 200 nos/ happa. After attaining 30 grams size ideally 5,000 Nos. can be stocked in a one acre pond.

Nutritional requirement & feeding

Pompano is a fast moving marine fish and it requires highly nutritive feed to meet the energy requirements. During nursery rearing Pompano can be weaned to any type of feeds viz., extruded

floating pellet, sinking pellet feed and chopped trash fishes. Ideally pompano can be weaned to extruded floating pellet feed to avoid feed wastage and spoilage of pond bottom. The CMFRI has conducted pompano farming demonstration by using the extruded floating pellet feed manufactured by M/s. Rudhra Techno Feeds, Bhimavar-am, Andhra Pradesh. During the happa rearing phase, feeding has to be done 4 times a day and in pond culture phase it could be 3 times a day. The feed size should be lesser than the mouth size of the fish and hence, suitable sized feed has to be selected for feeding the fishes. The details of feed and feeding schedule of pompano are as follows:-

Weight of the fish	Feed Size	Crude Protein %	Crude Fat %	% to be fed as per the biomass	Feeding / day
> 1 Gram	800 - 1000 μ	50	10	30	4
1 – 10 gram	1.0 - 1.5mm	40	8	20	4
10 – 100 gram	1.8 mm	35	8	8	3
100 – 250 gram	3.5 mm	30	6	5	3
250 – 500 gram	4.5 mm	30	6	3	3

A mix of two sizes of feed pellet can be used if there is any size variation of the fishes found during the regular sampling. If sinking pellet feed is used, at least 4 – 8 feed trays (80 cm x 80 cm) per pond could be placed. Regular sampling of fishes once in 15 days has to be carried out to determine growth rate and to calculate the FCR. In the first farming demonstration, FCR was 1: 1.8 with the above formulations.

Water Quality Management

Plankton bloom is essential for early stages of pompano (until 100 grams) culture. If the color of

the pond water is clear a mixture of organic (10-30 kg./ha.) and inorganic fertilizers (1-3 kg./ha.) can be applied to obtain algal bloom. Sufficient water level must be maintained in the ponds to reduce risks of the growth of benthic algae. The water depth in the shallowest part of the pond should be at least 100 cm. Water quality can be maintained by exchanging 10% of the water once in a week; 20% per week after 3 months and 30% per week after 6 months. If water colour is too dark, the quantum of water exchange can be proportionately increased. To maintain water pH within an optimum range of 7.5 - 8.5, agri-lime has to be

applied regularly. Dissolved oxygen (D.O) level should be maintained above 5 ppm at all times. Paddle wheel aerators can be placed in the pond to create minor water current and to maintain the DO level. Aeration is a must during late evening to early morning period when the fishes attains 200 grams size and above.

Growth Pattern

DOC	Growth (mm)	Weight (g)
1	30.59 ± 0.24	2.00 ± 0.04
30	73.42 ± 0.53	15.08 ± 0.16
60	102.88 ± 1.91	34.60 ± 0.41
90	158.39 ± 2.42	72.54 ± 1.95
120	182.30 ± 2.03	101.82 ± 3.11
150	203.71 ± 3.73	172.39 ± 4.55
180	226.51 ± 2.90	258.31 ± 5.76
210	273.07 ± 3.62	375.32 ± 8.07
240	296.88 ± 6.27	464.65 ± 10.25

During the entire culture period the growth pattern of pompano was monitored through regular sampling of fishes at fortnightly intervals. The length and weight measurements taken are presented below:-

Health management

Pompano is a much hardier species and does not get much disease problems. When it is reared in high salinities parasitic infection of copepods

may occur. Periodical application of commercially available pond management chemicals like Iodine solution would help to keep the fishes healthier. Feed supplements like LIV- 52 syrup can be given by mixing with the feed to improve the immunity levels.

Harvesting

Harvesting of pompano could be carried out by using drag net as in the case of fresh water fishes. To maintain the freshness and quality of harvested fish, washing in clean water and chill killing can be done. Harvested fishes can be stocked in plastic crates by adding layers of ice in equal quantities at the bottom and top of the fish. It is suggested that harvesting of fish can be carried out during the off season period of April to June to get a better price.

It is well recognized that for sustainable production in aquaculture, diversification of species is a vital requirement and from the lessons learnt from the shrimp farming scenario in India, it is very much needed to diversify the marine and brackish water aquaculture with high value fin fish species. Generally, high value marine fishes are in good demand in the Indian market and often there is a scarcity of the same. In the domestic market, silver pompano has demand starting from 250 grams size onwards. Hence, it is felt that pompano aquaculture can prove to be much lucrative and can emerge as a major aquaculture enterprise in the coming years.

Broodstock development of mangrove red snapper
Lutjanus argentimaculatus **in open sea cages**

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Introduction

The snapper family, Lutjanidae, belongs to the order Perciformes, the largest order of vertebrates, with 148 families and nearly 9,300 species. The Perciformes is a group of spiny-rayed fishes that are especially common in tropical and subtropical seas, and are usually found in coastal areas; however, it also includes a few families restricted to freshwater (Nelson 1944). The family Lutjanidae is composed of 17 genera and 103 species of mostly reef associated marine fishes, several deep-water (> 100 m) species and three freshwater species. The family is divided in four subfamilies. Three smaller subfamilies include the Paradicichthyinae with two monotypic genera (*Symphorus* and *Symphorichthys*), the Etelinae with five genera (*Aphareus*, *Aprion*, *Etelis*, *Pristipomoides* and *Rhandallichthys*) and 18 species, and the Apsilinae with four genera (*Apsilus*, *Lipocheilus*, *Paracesio* and *Parapristipomoides*) and 10 species (Allen, 1985). Several new species of snappers and even genera have been described recently (Anderson 1981, Akazaki 1983, Randall et al. 1987, Iwatsuki et al. 1993, Allen 1995); however, there is still debate within the scientific community about the validity of these species and biological information about them is extremely limited. The subfamily Lutjaninae represents about two thirds of the species in the family and it is the best known; however, the other three subfamilies also deserve attention and are relevant aquatic resources in many regions of the world. The species in the subfamily Lutjaninae constitute an important component of the reef fisheries in tropical and sub tropical latitudes throughout their geographical range, while the deep-water subfamilies Apsilinae and Etelinae represent by far the most important component of the deep-bottom fishery in Hawaii and other areas of the Pacific, Atlantic, and Indian oceans. The snappers are of economic value due to the excellent quality of the meat and high demand, making them some of the most appreciated species in the market today. However, there is concern about the status of several fish-

eries. In the Gulf of Mexico alone, red snapper (*Lutjanus campechanus*) and vermilion snapper (*Rhomboplites aurorubens*) are currently overfished (Coleman et al. 2000). Cubera snappers (*L. cyanopterus*) and mutton snapper (*L. analis*) are listed as vulnerable by the International Union for Conservation of Nature, and considered at risk of extinction (IUCN 2002). Decreases in natural populations of snappers have motivated new interest in developing techniques for reproducing them in captivity, either for fishery enhancement or for commercial cultivation. Research in this area has focused on understanding the life cycle and nutritional requirements of selected species to find technology for producing reliable sources of eggs and fingerlings (seed production) and to determine the best rearing conditions. Currently, *Lutjanus argentimaculatus*, *L. johnii*, *L. russelli*, and *L. sebae* are successfully farmed in floating net cages in Pakistan, China, Singapore, Malaysia, Thailand, and the Philippines (Doi and Singhagraiwan 1994, Emata et al., 1999, Hussain and Khatoon, 2000, Hong and Zhang, 2002). In the U.S.A., aquaculture research has been conducted on *L. campechanus*, *L. analis* and *L. griseus* among others (Watanabe et al., 2001, Chigbu et al. 2002).

In recent years, the aquaculture of the mangrove red snapper has become immensely popular in Southeast Asia and Australia due to its high market price. However, the culture of this species is still exclusively dependent on wild fry where the supply is limited, seasonal and unpredictable. This limits the sustainability of its aquaculture. Thus, a reliable breeding and fry production technique must be developed to ensure consistent production of good quality fry to meet the demand of the industry. Presently, the mangrove red snapper sexually mature and spawn spontaneously in concrete tanks and floating net cages at the Southeast Asian Fisheries Development Center's Aquaculture Department (SEAFDEC/AQD) (Emata, 1996; Emata et al., 1999). However, there are variations in egg and larval quality and larval survival in the hatchery remains poor (< 1%).

There is no information available on effects of broodstock nutrition regarding reproductive performance. This paper, therefore, is an initial study to develop a quality broodstock of red snapper to ensure consistent production of highest-quality eggs and larvae to support mass production of fry in the hatchery.

Establishing of cages for broodstock development in open sea

In this context, CMFRI established a broodstock development unit for *Lutjanus argentimaculatus* in the open sea at Chellanam area at Kochi, Kerala without affecting the traditional or mechanized fishing activities of local fisherman, where a highly productive area is available for mariculture activities. A circular fish rearing unit of 6 m diameter and a rectangular mini lab cum watchman's cabin of 6 x 3 m were made of 1.5" GI pipe (B class). The circular fish rearing unit was provided with height of 120 cm from base to the railings. All the joints are double welded to ensuring extra strength. After fabrication, both the structures were provided with three layer of FRP coating to prevent corrosion and ensure duration. Both the units were also provided with working platform with FRP resin coated plywood. To ensure sufficient buoyancy, 16 nos and 14 nos. of 200 l barrels filled with 30 lb air were used as floats for the rectangular and circular units respectively. The rectangular unit besides being used for watch and ward is also used for sampling, analysis of environmental parameters, cannulation, hormone treatment, growth analysis, etc. Fish rearing units are provided with one outer net made of HDPE Braided twine with 3 mm thread thickness/ mesh size of 80 mm fitted with 14 mm rope (6 m diameter and 8 m depth). The inner net is made of HDPE Twisted webbing with 1.5 mm twine thickness/ mesh size 28 mm fitted with 12 mm rope (5 m diameter and 7 m depth). The rectangular unit is also provided with an outer and inner HDPE nets of similar specifications as above of 3 m x 3 m x 5 m; l x b x d. For the circular unit, ballast pipes weighing 150 kg with 30 mm wire rope for the outer net and that weighing 125 kg with 18 mm wire rope for the inner net are provided. The units are provided with a stable platform with resin coated marine plywood where researchers can stand and safely attend works pertaining to handling of broodstock, net cleaning, net re-

placement, etc. The mooring is done with cement blocks for each unit and is connected to the GI structure using metal chains. The location of main block was documented using GPS and indicated with circular floats. The shock absorber was also set using 4 floats. The units are also provided with lightings in the night for indicating structure.

Food and feeding

The study of feeding habits is important because fish growth depends on the quality and quantity of food that is eaten. The question of how fish select their food was first addressed during the late 1960's (Emlen 1966) and led to the development of the optimal foraging theory which attempts to explain how an individual chooses between alternative sources of food by weighing the benefits and costs of capturing one possible prey over another. This theory, although not precise, has influenced studies of fish feeding ecology for the last 20 years (Gerking 1994). Fishes grow throughout their lives and this phenomenon is a major element in their life history that influences how optimal foraging theory applies to them. As fishes grow they should make adjustments in their foraging strategy reflected as changes in food quantity, size or other characteristics. The larval stage in fishes is less well developed than the young of other vertebrates, and its food intake with regard to size and variety is limited when compared to that of adults; therefore, one optimal foraging strategy is not a consistent feature throughout the life of a fish species, but it needs to be adjusted during ontogeny (Livingston 1988, Gerking 1994). Snappers are active predators, often characterized as opportunistic carnivores that feed mainly at night on a variety of items. Although fishes are dominant in the diet of most snapper species, other important prey include crustaceans (mainly crabs and shrimp), gastropods, cephalopods, and planktonic organisms, particularly pelagic urochordates. The larger, deep bodied snappers generally feed on fishes and large invertebrates (especially stomatopods and lobsters) on or near the surface of reefs; they are usually equipped with large canine teeth adapted for seizing and holding their prey (Allen 1985). Snappers occur and feed from the surface to depths of over 500 meters; however, the adults of several species are restricted to feeding in water deeper than 100 m deep. Diets of the mainly deepwater species are poorly known because of

the remote locations they inhabit and the loss of gut contents by regurgitation due to the expansion of the swimming bladder when a fish is brought to the surface (Parrish 1987).

Broodstock development

Wild collected red snapper juveniles of size ranging from 600 to 1200 g were stocked in the open sea cages. The fish were stocked without separating sexes. The collected snappers were dipped in 100 ppm formalin. The fish were fed twice daily at 0900 and 1530 hrs with sardines, mackerel, squid, *Stolephorus*, and mussel meat @ 5% body weight. Vitamin and mineral supplements were also given twice a week along with feed in order to complement any possible nutritional deficiencies in the regular diet. A total of 18 fish were stocked in the 6 m dia cage and 13 fishes in the square cage. The length and weight of fishes were recorded once in every 15 days. The sexes were separated by cannulation using a flexible catheter (2 mm inner diameter). Thereafter the females were cannulated every fortnight to assess the diameter of the intra-ovarian eggs.

Induced maturation of red snapper in open sea cages

The sub adults were induced for ovarian development with HCG at doses of 500 IU per kg body weight for female and 250 IU per kg body weight for males once in 30 days. The regulatory influence of the hypothalamus on the reproductive functions of the pituitary of fishes and their functional relationship with the pituitary, as well as the chemical nature of neurosecretory materials has been well established (Goos, 1978). The hypothalamus of teleost fishes comprises the Gormori-positive nucleus preopticus (NPO) and the Gormori-negative nucleus lateralis tuberis (NLT) (Sage and Bern, 1971; Peter, 1973; Holmes and Ball, 1974).

For the mangrove red snapper *L. argentimaculatus*, repeated injection of hormones have resulted in development of egg in the female fish. However, the process to obtain fully developed and matured egg and spawning thereafter is under standardisation. The open sea cage units are found ideal for broodstock development of red snapper due to the continuous exchange of good quality water and waste removal. Since the fish is high valued and has high fecundity (about one

million eggs) it is considered as the future candidate species for aquaculture. Other attributes are its fast growth rate, euryhaline nature, compatibility to grow in crowded conditions as in cages and low disease issues.

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Breeding and seed production of Clown fishes under captivity

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Introduction

Ornamental fish production for the aquarium industry is a multimillion dollar industry in the world. Aquarium keeping is amongst the most popular of hobbies with millions of enthusiasts worldwide. Although most fish kept in aquariums are from freshwater, the acquisition of marine ornamental fish has greatly increased in recent years and is also popularized through children's movies by starring charismatic colourful fishes and other creatures. Recent advances in fish husbandry and aquarium gadgets and technology have further facilitated the hobby. In India, marine ornamental fish trade is an emerging area during the last two decades and the industry is further advancing. Along with it ancillary business such as aquarium making, aquarium plants and live feed production, grow-out culture etc. are also flourishing.

India has about 200 varieties of marine ornamentals, of which more than 50 have export potential. Among these, the clown fishes or anemonefishes belonging to the family Pomacentridae, comprising of genera *Amphiprion* and *Premnas* have always been the most popular and sought after group. Altogether 28 species of clown fishes were reported from the different geographical locations of the world. The members of the family Pomacentridae commonly known as damselfishes and anemonefishes are a diverse group of marine fishes found in tropical seas, also have very high demand in marine ornamental fish trade. The family comprises 29 genera and 350 species inhabiting in the coral reef ecosystems. Pomacentrids have been divided into four subfamilies: Amphiprioninae, Chrominae, Lepidozyginae and Pomacentrinae. Under the genera *Amphiprion*, 29 species and a single species, the maroon clown *Premnas biaculeatus* have been reported under the genus *Premnas*.

Anemone fishes or clown fishes

The genera *Amphiprion* and *Premnas* belonging to the family Pomacentridae and sub family Amphiprioninae commonly known as "clown

fishes or sea anemone fishes" are long ranked as one of the most popular attractions all over the world because of their tiny size, hardiness, attractive colour features, peaceful nature, high adaptability to live in captivity, acceptability to artificial diet and their fascinating display behavior and symbiotic relationship with the sea anemones. Clownfishes are the most longstanding and intensively cultured family of marine ornamentals and are the best ranked in marine aquarium trade. They were the first reef fish species bred successfully in captivity. However, large scale culturing of clownfish has not always been successful technically due to the lower larval survival which usually go through one or many larval stages, start out at a very small size, and are extremely sensitive to external factors. The clown fish species most studied is *A. ocellaris*. The technique used for this fish has been used for a long time to establish the protocol or guidelines for the breeding of other clownfish such as *A. chrysopterus*, *A. clarkii*, *A. percula*, *A. melanopus* and *Premnas biaculeatus*.

From the Indian waters 15 species in the genera *Amphiprion* and the single species of the genus *Premnas* have been reported (Madhu and Madhu 2000). Most of the traded marine ornamental fishes are being collected from the wild and hence there is a great concern regarding the depletion of the stocks due to over exploitation as well as the destruction of reef habitat and damaging collection methods all over the world. Recent studies shown that captive bred clownfish are generally hardier, disease free, and are better adjusted to life in aquaria than their wild-collected specimens, and as a result the demand for the captive bred fishes are increasing. For the breeding of clown fishes under captive conditions, few important steps are to be followed. These include selection of suitable broodstock, setting up the tank, broodstock feeding, maintenance of high water quality, provision of suitable environmental parameters, creating suitable condition for spawning and system for raising the larvae and juveniles.

Transportation of broodstock

For the captive mass production of clown fishes, the basic requirement is to have a sufficient number of broodstocks or breeding pairs which can either be collected from the coral reef habitat or can be purchased from the pet shop depending upon the availability. In the wild, the clown fishes generally occupy in social groups centered in a host sea anemone with a sexually active pair of adults and one to three juvenile or sub adult fish and the female is larger than the male. The clown fishes naturally exhibit monogamous pair formation and these pairs are to be collected for broodstock development and breeding programme. In case such mated pairs are not available, fish of different size groups can be collected and allowed to form pair under captive conditions. In order to make breeding pairs from the juveniles groups, many social groups of clown fishes can be collected from the wild and transported to the laboratory. During transportation, the fish and the sea anemones should be kept in separate transportation containers.

Pair formation

For pair formation, five fishes of each sex of different size groups need to be stocked together along with a host anemone in a 500 L FRP tanks fitted with biological filter to reduce the aggression. Pair formation tanks have to be maintained in the hatchery with a light intensity of 2500 to 3000 lux as the anemones require light for survival under laboratory conditions. The fish and anemones should be fed twice a day with wet feeds like shrimp, mussel and clam meat at the rate of 15% of their body weight and live feeds like *Brachionus plicatilis*, *artemia nauplii* and adult *artemia*. Environmental parameters such as temperature (26 to 29°C), salinity (33 to 35 ppt), dissolved oxygen (4.6 to 6.2 ml/L) and pH (8.1 to 8.4) are to be maintained in all rearing tanks.

Sex change and pairing

As the clown fishes are protandrous (male first) sequential hermaphrodites, a pecking order is established in which the female is dominant, the male is subordinate to the female, and all the other juveniles are subordinate to the adult male and female. Thus generally all clown fish individuals start out as males and change into females when they reach larger sizes or under the situation of loss of mate. The male and female form a monogamous pair bond that lasts until one member

of the pair dies. If the female dies first, the largest male rapidly changes sex into a female and the second largest or dominant juvenile becomes an active male and that pairs up with the newly transformed female. By utilizing this adaptation, pairs of clown fishes can be developed under captive condition by creating social systems. After a period of 3 to 4 months of rearing for pair formation, in each tank one pair grew ahead of others and becomes the spawning pair. As the newly formed pairs will be very aggressive and spending time for fleeing the other subordinates rather than reproductive activity, it is very essential to stock each breeding pairs in separate tanks.

Tank set-up for broodstock

A clownfish broodstock/ spawning tank should be of 250 to 500 L capacity with a single healthy pair and host sea anemone. An ideal tank would be a 3 ft x 2 ft x 2 ft with a layer of coral sand at the bottom, few live rocks, bright lighting and good filtration, preferably with an efficient protein skimmer to reduce the ammonia and organic materials in the system. A trickle filter could be used with regular water changes to keep the nitrates low enough for the anemone to do well. Since the gonad development and spawning of clown fishes are influenced by moon phases, the broodstock/ spawning tanks should be kept at a place where the fish receive regular day/ night light cycle (moon phase). Anemone is generally not required to breed clownfish under captive condition. But generally the clown fish select a nest site adjacent to the sea anemone for deposition of eggs. Moreover an added benefit of having an anemone is that it may release compounds that help to protect the eggs or even chemically induce immunity that clownfish have with the anemone.

Broodstock development and maintenance

After the pairs are formed, they are transferred to glass aquaria for broodstock development. Depending upon the production capacity and seed demand, several pairs can be maintained in commercial hatcheries. The broodstock are fed with meat of green mussel, shrimp, clam and fish egg, along with supplemental formulated feeds enriched with vitamins, minerals and algal powder at the rate of 10% of their body weight and fed during day time at an interval of 3 h. Apart from these, the broodstock are also fed with enriched rotifer 800 to 1000 nos/ml and *artemia nauplii*

(200-400 nos/ml) and adult artemia (3 to 5 nos/ml) every day.

Enrichment of rotifer and artemia

Three litres of enrichment medium has to be prepared using microalgae (*Chlorella salina*, *C. marina*, *Nannochloropsis oculata* @1x10⁶ cells/ml) for enrichment. To this, an emulsion prepared with homogenized cod liver oil (5 g), vitamin A (0.1%), vitamin D (0.2%), vitamin E (0.3%) and vitamin K (0.1%) has to be added. The rotifers are released to this enrichment medium @ 800 to 1000 nos/per ml along with 50 mg/L of bakers' yeast for 12 to 24 h. The rotifers thus enriched are harvested, washed and used for feeding the fishes.

The artemia nauplii (instar II stage) harvested through 100 μ bolting silk cloth after completion of about 16 hours of hatching, are released @200-400 nos/ml to the 5 L plastic circular tub containing 4 L of mixed algal water: *N. oculata*, *P. lutheri*, *C. marina* and *C. salina* (105 cells/ml), *I. galbana*, *D. inornata*, *C. pleoides* (104 cells/ml) for bioencapsulation and maintained at optimum environmental parameters. To this, 8 g of cod liver oil with fat soluble vitamin: vitamin A(0.2%), vitamin D(0.1%), vitamin E (0.6%) and vitamin K (0.3%) are added. The enriched artemia are harvested and fed to the fish after 12 to 24 h of enrichment.

Feeding with enriched live feeds

After enrichment, the rotifers and artemia were harvested, washed and released to 4 L of bio-filtered seawater containing mixed culture of microalgae: *N. oculata*, *P. lutheri*, *I. galbana*, *D. inornata*, *C. pleoides* and *C. marina* (104 to 106 cells/ml) in 5 L capacity transparent tub with mild aeration. The enriched rotifer and artemia were given in split dose (10-11am and 3.0 to 4.0 pm daily).

Water quality maintenance

Maintenance of water quality is the most critical factor for breeding of clown fishes or any marine fishes under controlled conditions. As a measure for this, the sea water need to be filtered through a series of sand filters before being taken to the rearing tanks. The temperature in all the breeding tanks has to be maintained between 26 to 30°C, dissolved oxygen (4.8-6.3 ml/L), pH (8.0-8.4), salinity (32-35 ppt) and the water should be recirculated to ensure water movement and good quality during the rearing period. Once in a week 25%

of the water should be exchanged to avoid stress like a rapid increase in plasma cortisol concentration, depression of gonadal steroidogenesis, and subsequent development of gonadal atresia.

Egg deposition

The clownfish have attached eggs and are known to spawn on rough surfaces near the host anemone. Hence it is essential to provide suitable substratum preferably tiles or earthen pots or shells of edible oyster or PVC pipes for the egg deposition which will also be helpful for the transfer of deposited egg to hatching tank without any mechanical injury.

Breeding behaviour

After broodstock rearing, each pair will start breeding within a period of 4 to 6 months under captive condition. Few days prior to spawning, the male select a suitable site near to the anemone for egg laying and it clears algae and debris with its mouth and on the day of spawning both the parents spent considerable time for cleaning of the site which indicates the imminent at within few hours. Under laboratory conditions, the spawning can be obtained between 0500 h to 1530 hrs during day time and it lasts for one to one and a half hours. Each female lays 300 to 1000 capsule shaped eggs at every 12 to 15 days interval depending on the species and size of the fish. The egg size ranges between 1.5 mm and 3.0 mm in length with a width of 0.8 to 1.84 mm and remain adhered to the substratum with a stalk. At an average two spawnings/ pair/ lunar month result in an estimated annual fecundity of 7200 to 24000 eggs/ breeding pair/ year under laboratory conditions.

Parental care

As parental care is inevitable for hatching out of the larvae, the parents should be allowed to remain in the tank itself till hatching. During incubation period, both the parents carefully look after the eggs during day time and it involved two basic activities viz. fanning by fluttering the pectoral fins and mouthing to remove the dead or weakened eggs and dust particles. The newly spawned eggs are white to bright orange in colour for initial two days and as the embryo develop; these are turned to black on days 3 to 6 and later to silvery on day 7 to 8 of incubation. At this stage the

glowing eyes of the developing larvae inside the egg capsule is clearly visible when viewed from a short distance. Male assumes all the responsibility of caring for the eggs and spent a higher percentage of time at the nest than the females, which then increase gradually up to 70% of time as the day of hatching approaches. When incubated at 27 to 29° C, hatchling was emerged on day 8 and the peak hatching took place shortly after sunset.

Egg hatching and larval rearing

On the expected day of hatching, two hours before sunset, the eggs along with substratum were transferred from the parental tank to hatching tanks (100 L) and provided with complete darkness for accelerating the hatching. The larvae broke the egg capsule and the tail of the hatchling is emerged first and the hatching occurred soon after sunset and the peak hatching took place between 1900 to 2030 hrs. The newly hatched larvae measured 3 to 4 mm in length with transparent body, large eyes, visible mouth, and a small yolk sac and remained at the bottom of the tank for a few seconds and soon after became free swimming. The larval rearing was carried out under green water system and feeding with super small rotifer *B. rotundiformis* and newly hatched artemia nauplii. The larval period of clown fishes generally last for maximum of 20 days and then after most of the fry resembled juvenile adult fish and began to shift from partially pelagic to epibenthic and started eating minced shrimp, fish flesh, musselmeat, clam meat and formulated diets.

Larval feeding

The successful feeding strikes are low at first feeding but rises rapidly during early development in clown fishes. At this stage provision of suitable size and nutritionally adequate enriched feed in high density is important for their survival. Larvae have only little quantity of yolk material and it starts feeding within few hours after hatching. As the mouth gape of clown fish larvae is between 80-123 μ , the larvae need to be fed with live feeds measuring less than 100 μ for its active feeding. The rearing tanks need to be provided 24 hrs with light up to 15 days of post hatch (DPH). During this time the larval tank must be kept very clean with the bottom siphoned off by removing dead larvae, detritus and faeces twice a day from the bottom. Water exchange has to be done at a rate of at least 25% per day.

Feeding schedule of larvae of clown fishes can be performed in two stages: Stage 1: covered the rotifer with algae feeding phase from Day 1 to day 8; Stage 2: the newly hatched artemia and rotifer with algae feeding phase from day 9 to day 20. For the successful prey capture of larvae, 50-100 numbers ml-1 rotifer (*B. rotundiformis*) of 60 to 100 μ size need to be provided after enrichment with vitamins and fatty acids. As the larvae attains successful prey capture within two days, the density of rotifer in the larval rearing tank need to be reduced to 30-50 nos. ml-1 from day 3 to 8. From day 9 onwards the larvae were weaned onto newly hatched Artemia nauplii (5-10 nos/ml) along with rotifer (SS and S type) (20-30 nos/ml) whereas algal concentration should be same as the prey capture step till day 20 of post hatch. The clownfish have a larval period between 10 and 20 days. After 20 days of rearing the larvae develop the adult striped colouration and metamorphose to juveniles and shift from partially pelagic to epibenthic and look like miniature adults. From metamorphosis onwards, the clownfish actively swim on the bottom of the tank and settle in the host sea anemone. Up to 20 days, the rearing can be carried out in the same tank and on completion of metamorphosis, the juveniles should be graded into several groups and stocked in separate tanks in which biological filtrations system need to be was provided.

Copepod as a live feed

Survival can be significantly made higher when larvae were fed with copepods. The higher omega-3 fatty acids found in copepods appear to be important for survival of larvae under more stressful conditions. But mass production of copepod is often collapsed due to several factors. Hence dependence on copepod for larval rearing is unreliable until and unless a copepod mass production technique is standardized.

Rearing conditions

The maintenance of water quality is a critical factor in larval rearing of clown fishes or any marine fishes under controlled condition. As a measure for this, the sea water needs to be filtered through a series of sand filter tanks before being taken to the larval rearing tank. However during larval rearing it was found that the period from 3rd to 8th day of post hatching (dph) was very critical may be due to the alteration or change

in feeding (exogenous) whereas once the larvae completed 8 days after hatching, no further mortality was observed. During the larval rearing period, in all tanks, the environmental parameters were maintained to their optimum level with pH ranging from 8.0 to 8.4 water temperature 26 - 30° C, dissolved oxygen 5.5 - 7.8 (mg/L), salinity 33-35 ppt, NH_4^+ / NH_3 and NO_2 values at 0 mg per L and NO_3 levels below 0.2 mg /L. Daily the tanks were cleaned with cotton and magnetic tank cleaner to remove the dust and slimy coating forming inside the tank and one fourth water is replaced with same amount of filtered sea water along with enriched rotifer and artemia and micro algae.

Light intensity

Head-butting syndrome was another the critical problem encountered during larval rearing due to the immature development of the retina and subsequent hitting of larval head to the sides of the tank. In order to reduce this, two major measures taken were i) all the 4 sides of the tanks were covered with black cloth or painted black to avoid reflection of the light ii) a low intensity light provided by hanging 2 nos. of 60 watt bulb or night lamp at a height of 15-20 cm from the surface of water level in rearing tank for 24 hours from day 0 to day 20 which enable the larvae to detect and capture its feed and it also helped them to swim towards the surface at night rather than sinking to the bottom which otherwise show high overnight mortality. The type of lighting is not critical and can be from any source of light, i.e. fluorescent or metal halide etc. The reason for having a light is that the larvae are visual predators and require light to hunt for their live food. In addition to these, all the larval tanks need to be covered with net cloth during the night time to prevent the entry of insects.

Problems in larval feeding

In general, the mortality of larvae were reported due to over eating, intestinal blockage, ingestion of air bubbles or bacterial problems. Though Artemia is in regular use for larval rearing of marine fishes, there is one serious concern with introducing unhatched cysts along with the Artemia nauplii to the larval rearing tank and these cysts are often eaten by the larvae and will cause intestinal blockage. Hence care must be taken to separate all the empty cysts from the newly hatched

artemia before being added to the larval tanks. It is also equally important to add newly cultured or hatched live food every day because the nutritional value of the live food that remains in the tank will decrease very quickly. The nutritional quality of rotifer also depends upon the quality of feed offered. Hence every day, after water exchange from the larval rearing tanks, new rotifers and or Artemia must be added. The healthy larvae will appear to have a round body and swimming in a close horizontal position. Unhealthy larvae will tend to either buzz around on the surface at 45 degree angle. For the first two days there will be some loss of larvae, if the larvae have been transferred using the siphon method. From day three to eight the larvae will grow very fast. The densities of live feed can be reduced as the larvae have become proficient at food capture.

Juvenile rearing

On days 19-20 of post hatch, the larvae became juvenile and shift from pelagic to epibenthic stages, and look like miniature adult fish. The rate at which the young fish grow depends on the size of the rearing tank, stocking density, quality and quantity of food given and the water temperature. As the clownfish exhibit social hierarchy, dominant clownfish will grow faster and will suppress the growth of the fish below. However, this can overcome by growing the fish altogether in a large tank with sufficient host anemones or culling the juveniles to several groups in different juvenile rearing tanks of 250 to 1000 L capacity. At this stage, the stocking density has to be reduced to 90 -100 numbers of juveniles (size range between 8-10 mm) with single host sea anemone in glass or perspex tank at 100 L capacity for initial 1 to 2 months rearing. During juvenile stages, the fishes show different banding pattern and growth rate, and on attaining a size of 24 to 35 mm in total length (TL), the stocking density need to be reduced to 30 to 50 number with single sea anemone in 100 L tank with 80 L bio filtered sea water until marketing. In the case of each 500 L FRP tanks, 130 to 150 juveniles can be reared with 1 to 3 anemones.

Feeding

In the juvenile rearing, a survival of 100% was obtained through feeding with different wet feeds at the rate 15 to 20% of body weight. Apart from these, artemia nauplii 10-15 numbers/ ml and ro-

tifer (*B. plicatilis*) 50- 55 nos./ml were given after enrichment with brown algae (104 cells/ml) and green algae (106 cells/ml) with cod liver and fat soluble Vitamin A, D, E, K, twice a day which helped to retain the colour of fishes and provided adult artemia (2-4 nos/ml). Through this feeding schedule, the larvae will attain 10 to 12 mm within 30 days of post hatch and the juveniles reach 25 mm to 35 mm within 60 days and attain marketable size within 6 months after post hatch. Once in a week, one third water need to be decanted and refilled with same quantity of filtered sea water in all juvenile rearing tanks. With these feed management procedures, 90-95% of larval survival can be obtained under captive conditions in each spawning.

Packing and Transportation

Fishes are starved for about 2-3 days before being exported. A small amount of freshwater is added to the packing water and chemicals may be added to tranquilize for longer journeys. Packing starts just prior to the transportation. Fishes are packed with oxygen and a little water either singly in double polythene bags to ensure that fish are not stranded without water. Polythene bags are packed in cardboard boxes for short journeys and for long journeys they are packed in Styrofoam boxes with some ice to keep the temperature down. Layers of paper may be inserted between plastic bags in the box to avoid catching sight of aggressive species. Packaging methods have improved considerably over the years mainly due to feed back from the customers and many exporters now guarantee almost 100% survival for most destinations provided that good connecting flights is available. Regulating the standards of the holding facilities and of standards of packing is important to ensure minimum mortality of fish at holding facilities and in transport.

Marine ornamental fishes bred in India

Central Marine Fisheries Research Institute (CMFRI) has taken initiatives on culture of marine ornamental fishes with objectives to generate scientific knowledge on ornamental fish maintenance, behaviour, influence of social status on

sex change, pair formation, breeding, influence of lunar periodicity in spawning, parental care, egg incubation and hatching, developments of egg, larvae, and juveniles. These investigations have resulted in the development of hatchery technology for 20 species of marine ornamental fishes such as clown fishes True pecula/ clown anemone fish *Amphiprion percula* (Madhu and Rema, 2000,2002); Common Clown/ False clown anemone fish *A. ocellaris* (Rema et al.,2012); Yellow Skunk Clown *A. sandaracinos* (Rema and Madhu, 2012); Tomato clown *A. frenatus* (Madhu et al, 2011), Clark's Anemonefish *A. clarkii*, Maldives Anemonefish *A. nigripes* (Madhu and Rema Madhu,2006; Madhu et al., 2006a,b,c; Rema Madhu, et al., 2007; Madhu et al., 2008, Madhu and Rema , 2011), Pink anemone fish *A. perideraion* (Anil et al.,2012), redsaddle back anemone fish *Amphiprion ephippium*, Sebae clown *A. sebae* (Gopakumar, et al.,2007, 2009); and Maroon clown/ Spine cheek anemone fish *Premnas biaculeatus* (Madhu et al., 2012) and dotty back *Pseudochromis dilectus* (Redhead Dottyback) were bred. The species such as damsels Three spot damsel *Dascyllus trimaculatus*; Striped damsel *D. aruanus*; Blue damsel *Pomacentrus caeruleus*; Sapphire or Peacock Damsel *P. pavo*; Yellow tail damsel *Neopomacentrus nemurus*; Filamentous tail damsel *N. filamentosus*; Sapphire devil *Chrysiptera cyanae*; One spot damsel *C. unimaculata* and Green chromis *Chormis viridis* (Gopakumar, et al.,2007,2009, Syda Rao et.al., 2010) for the first time in India.

Conclusion

Considering the commercial importance of anemonefishes, it is very essential to develop the breeding techniques for mass scale production under captive condition. In order to produce its seeds, healthy broodstocks need to be reared for pair formation and breeding. As the clown fishes are protandrous and breed two times per month, provision of suitable feed and maintenance of environmental parameters are the important management practices for obtaining consistent breeding under captivity.

Seed production and culture of marine ornamental fishes

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Introduction

The marine ornamental fish trade has been expanding in recent years and has grown into a multimillion dollar enterprise. The ornamental animals are the highest valued products that are mostly harvested from coral reef environments. The global marine ornamental trade is estimated at US\$ 200-330 million. The trade is operated throughout the tropics. Philippines, Indonesia, Solomon Islands, Sri Lanka, Australia, Fiji, Maldives and Palau supplied more than 98% of the total number of marine ornamental fish exported in recent years. It is a multi-stakeholder industry ranging from specimen collectors, culturists, wholesalers, transshippers, retailers, and hobbyists to researchers, government resource managers and conservators and hence involves a series of issues to be addressed and policies to be formulated for developing and expanding a sustainable trade. It is well understood that a long term sustainable trade of marine ornamental fishes can be developed only through the development and commercialization of hatchery production technologies for the species which are in high demand in the trade.

Global scenario

In recent years it has been reported that nearly 1500 species of marine ornamental fishes are traded globally and most of these are associated with

coral reefs. Nearly 98% of the marine ornamental fishes marketed are wild collected from coral reefs of tropical countries. Among the most commercially traded families of reef fishes, family Pomacentridae dominate, accounting for nearly 43% of all fish traded. The family contains about 235 species worldwide. They are followed by species belonging to Pomacanthidae (8%), Acanthuridae (8%), Labridae (6%), Gobiidae (5%), Chaetodontidae (4%), Callionymidae (3%), Microdesmidae (2%), Serranidae (2%) and Blennidae (2%). In recent years the blue green damselfish (*Chromis viridis*), the clown anemone fish (*Amphiprion ocellaris*), the whitetail Dascyllus (*Dascyllus aruanus*), the sapphire devil (*Chrysiptera cyanea*) and the three spot damsel (*Dascyllus trimaculatus*) are among the most commonly traded species.

Hatchery production technologies

Indiscriminate exploitation of ornamental fishes from the coral reef areas has been threatening the long term sustainability of the trade. Hence hatchery production of selected marine ornamental fishes is the only option for the development of a long term sustainable trade. The Central Marine Fisheries Research Institute (CMFRI) has been focusing on this vital aspect for the past few years. The Institute was able to develop hatchery production methods of the following species of ornamental fishes which are in high demand in the international trade.

- | | |
|---------------------------------|---|
| 1. <i>Amphiprion percula</i> | - Orange clown |
| 2. <i>A. ocellaris</i> | - False clown |
| 3. <i>A. sebae</i> | - Sebae clown |
| 4. <i>A. nigripes</i> | - Maldive's clownfish |
| 5. <i>A. ephippium</i> | - Red saddleback clownfish |
| 6. <i>A. perideraion</i> | - Pink skunk |
| 7. <i>A. clarkii</i> | - Clark's anemonefish |
| 8. <i>Premnas biaculeatus</i> | - Maroon clown(spine cheek anemonefish) |
| 9. <i>Pomacentrus cearuleus</i> | - Blue damsel |

- | | | |
|-----------------------------------|---|-------------------------|
| 10. <i>P.pavo</i> | - | Peacock damsel |
| 11. <i>Dascyllus trimaculatus</i> | - | Three spot damsel |
| 12. <i>Dascyllus aruanus</i> | - | Humbug damsel |
| 13. <i>Chromis viridis</i> | - | Bluegreen damsel |
| 14. <i>Neopomacentrus nemurus</i> | - | Yellowtail damsel |
| 15. <i>N.cyanomos</i> | - | Filamentous tail damsel |
| 16. <i>Chrysiptera cyanea</i> | - | Sapphiredevil damsel |

Clownfishes

Success was obtained in the seed production of eight species of clownfishes which are in good demand in the international trade of marine ornamental fishes

Amphiprion ocellaris

The spawning time was during early morning hours and the frequency of spawning ranged from 12 to 15 days. The clutch size per spawning ranged from 300 to 1000 eggs. Hatching was on the evening of 8th day of incubation and the newly hatched larvae measured from 3.2 to 4.0 mm in length. The larviculture protocols were developed and during the 15th to 17th day of hatching the larvae metamorphosed into juveniles.

Amphiprion percula

The spawning was during day time (0600 -1530 hrs) and the spawning interval ranged from 14 to 18 days. The clutch size per spawning ranged from 112-557 eggs. The hatching was on the evening of the 8th day of incubation and the length of the newly hatched larvae ranged from 1.91 to 2.02 mm. The larviculture protocols were developed and during the 19th -20th day of hatching, the larvae metamorphosed into juveniles.

Premnas biaculeatus

The broodstock was developed in 500 litre FRP tanks fitted with biological filtration and by providing special broodstock feeds. The spawning was during day time. The number of eggs per spawning ranged from 150 to 1000 numbers and the spawning interval was 15 to 20 days. Hatching occurred on the evening of the 6th day of incubation. The newly hatched larvae measured from 350 to 410 μ . Greenwater technique was

employed for larval rearing and feeding protocols with enriched rotifers and newly hatched *Artemia* nauplii were developed. At 15 to 17th day of post hatch, the size of the juveniles ranged from 12.0 to 16 mm.

Recently success was also obtained in the breeding and seed production of five more species of clownfishes viz. *Amphiprion nigripes*, *A. perideraion*, *A. frenatus*, *A. ephippium* and *A. clarkii*. The breeding and seed production techniques are similar to the species mentioned above.

Damsel fishes

Broodstock development and larval rearing were achieved for six species of damselfishes viz. the three spot damsel (*Dascyllus trimaculatus*), striped damsel (*Dascyllus aruanus*), the blue damsel (*Pomacentrus caeruleus*), the bluegreen damsel (*Chromis viridis*), the yellowtail damsel (*Neopomacentrus nemurus*) and the sapphire devil damsel (*Chrysiptera cyanea*).

Dascyllus trimaculatus

The mature fish ranged in total length from 9-10cm. The clutch size in a single spawning ranged from 12000 – 15000 eggs. The average periodicity of spawning was two weeks. The average length of newly hatched larva was 2.5mm. The green water technique with sufficient nauplii of copepods was the key factor for the success of early larval rearing. The larvae started metamorphosing from 35th day of hatching and all larvae metamorphosed by the 40th day. The just metamorphosed young one measured from 12-13mm in length. The second generation matured and spawned in the hatchery at eleven months of age.

Dascyllus aruanus

The brooders ranged in length from 7-8 cm.

The clutch size in a single spawning ranged from 8000 – 10,000. The average periodicity of spawning was two weeks. The average length of newly hatched larva was 2.4 mm. The larvae started metamorphosing from 25th day of hatching and all the larvae metamorphosed by 31st day.

Pomacentrus caeruleus

The breeders ranged in length from 7-9 cm. The clutch size in a single spawning ranged from 5000-6000 eggs. The average periodicity of spawning ranged from 3 to 12 days. The average length of the newly hatched larvae was 1.2 mm but the mouth gape was comparatively larger (around 200 μ). Greenwater technique and feeding with sufficient nauplii of suitable copepods for the first ten days and thereafter with freshly hatched Artemia nauplii was the methodology followed. The larvae started metamorphosing from the 17th day and by 21st day all of them metamorphosed. The average length of just metamorphosed juvenile was 21mm.

Chromis viridis

The broodstock development of the green damsel *Chromis viridis* was carried out in 2 tonne FRP tanks fitted with biological filter and by feeding with special broodstock feeds. The fishes became broodstock at a total length range of 8 -9 cm. The average frequency of spawning was 5 per month with an interval of about 5 days. The egg was oval shaped and the average length was 502 μ . The total numbers of eggs per spawning ranged from 1300 -1500 eggs. Hatching occurred on the evening of the fourth day of incubation. Larvae were altricial type with no mouth opening at the time of hatching. The average length of newly hatched larva was 2.25 mm. The larvae were transferred to 5 tonne capacity round FRP tanks in which cultures of the harpacticoid copepod *Euterpina acutifrons* and the calanoid copepod *Pseudodiaptomus serricaudatus* were maintained in green water produced by adding *Nannochloropsis* culture. Mouth opening was formed on the second day of hatching and the gape measured around 190 μ . The larvae started feeding on copepod nauplii from the 3rd day onwards. From the 32nd day of larval rearing freshly hatched Artemia nauplii was also supplemented. Metamorphosis started from 30th day and completed by 49th day.

Neopomacentrus nemurus

The broodstock of the yellowtail damsel *Neopomacentrus nemurus* was developed in 2 tonne capacity FRP tanks. The average interval of spawning ranged from 4 -5 days. The length of freshly laid egg was 870 μ . The eggs hatched on the evening of the fourth day of incubation. The freshly hatched larva measured 1.8mm with a mouth gape of about 100 μ . The larvae were transferred to 5 tonne capacity FRP tanks in which mixed culture of copepods were maintained in green water produced by adding cultures of *Nannochloropsis*. The larvae started feeding on nauplii of copepods from the third day of hatching. From the 12th day onwards the larvae were also fed ad libitum with freshly hatched Artemia nauplii. From the 16th to 21st day of hatching the larvae metamorphosed into juveniles. The length of the just metamorphosed juvenile ranged from 10 -13 mm.

Chrysiptera cyanea

Broodstock development was done in two tonne capacity FRP tanks with biological filter and by feeding ad libitum with natural feeds. The size of broodstock fish ranged from 5 to 6.5cm. The number of eggs per spawning ranged from 2000 - 2500. The interval between successive spawnings ranged from 5 to 20 days. The eggs were either attached to the sides of the broodstock tank or on the substratum provided in the broodstock tank. The eggs were oval - shaped and measured around 1.3mm in length and 0.6 mm in width. Parental care by the male was noted. Hatching occurred on the night of the third day of incubation. The larvae were altricial type but with mouth opening at the time of hatching. The length of newly hatched larvae averaged to 2.5mm and the mouth gape around 150 μ . Larviculture was done in five tonne capacity FRP tanks by employing greenwater produced by the microalgae *Nannochloropsis oculata*. Different larviculture systems were experimented by varying the cell counts of greenwater and the live feeds. The cell counts of green water employed for the experiments were 1 x 10⁴ ml⁻¹, 1 x 10⁵ ml⁻¹ and 1 x 10⁶ ml⁻¹. Four sets of experiments were conducted by feeding with different live feeds – one set with enriched rotifer (*Brachionus rotundiformis*) alone, the second set by employing mixed culture of two copepods species viz. *Euterpina acutifrons* and *Pseudodiaptomus serricaudatus*, the third set

by employing copepods and rotifers together as live feed and the fourth set with copepods as starter feed for the first six days followed by enriched rotifers from 7 -15 dph. The larval survival was recorded on 15th day of post-hatch. Feeding experiments with *B. rotundiformis* alone and those with *B. rotundiformis* and copepods together as live feeds were not successful. Co-culturing of the two selected species of copepods in the optimum range of cell count of greenwater gave the best survival. In this set, survival rate of larvae on 15 day post-hatch (dph) ranged from 5 to 8%. The maximum survival rate was 5-6% in the group fed with copepods as starter feed upto 6 dph followed by enriched rotifers from 7 to 15 dph. It was noted that a cell count range of 1×10^5 cells ml⁻¹ was the optimum which yielded the maximum larval survival in both these sets of experiments. After 15 dph the larvae were fed with freshly hatched *Artemia nauplii* and no further mortality was noted. Metamorphosis of larvae started from 24th day and all the larvae metamorphosed by 30th day.

The larviculture protocols of the other species are similar to the above.

Grow- out methods

Grow out of ornamental fishes can be effectively practised in happas installed in nearshore areas. The growth was found to be much faster. The major advantage is that the colour is much brighter in fishes grown in happas due to natural light and good exchange of water. The site for installation of happas should have at least 2 m depth of water, good dissolved oxygen content, free from industrial contaminants, low anthropogenic pollution and easy accessibility from land. A protected area is generally preferred.

Construction of floating hapa

Rectangular shaped fixed floating happa (2.5 m x 1.5 m x 1.5 m) with PVC frames (dia 1.5 inch) for supporting the net bag structure and to retain the shape are used for the grow out phases of juvenile to marketable size. Here the advantage is that it provides better water exchange and natural environment to the fishes.

Good quality HDPE net having 0.5 mm and 1 mm mesh size could be used to make the net bag. Double layered net bags are stitched in 2.5

x 1.5 x 1.5 m depending upon the design and requirement of the frame. Nylon thread is used for stitching the cages. Nylon rope (6 mm dia) is used for tying the bags and poles. All the joints are reinforced with nylon ribbon (1-1.5"). Ribbon loops are provided at regular intervals (0.5 m) both on the upper and lower margins of the hapa for tying the sinkers at the four corners with nylon rope. The top of the hapa is also covered with net frame. Two opposite corners of the top cover of the hapa is made detachable so as to enable regular feeding, growth monitoring and harvesting.

Survival of 90-95% is obtained through proper feeding with different wet feeds like boiled sardine flesh, chopped clam meat, mussel meat and formulated dry feed, two times a day ad libitum. Since the hapa was installed in the sea, fouling was a regular phenomenon and regular monitoring is advisable. Cleaning the net with coir brush has to be carried out on daily basis. Checking of the outer and inner net was also recommended on daily basis to detect any defects in nets. In addition to this, checking of mooring system twice in a week is advisable. Hapa reared marine ornamental juveniles grow faster with increased survival rate and good colouration thereby juvenile fetching better price in the market.

Feeds

For feeding marine ornamental fish CMFRI has scientifically evaluated feeds containing not less than 30 % protein, 9 % fat, 39 % carbohydrates, 7 % ash (minerals) and less than 2 % fiber. These feeds are made up of marine protein, soy protein, wheat flour, oil, vitamins, minerals, color imparting nutrients, immune promoters, an anti-oxidant, antifungal and probiotics. They are sold in packets of 50 g capacity. Technology commercialization package is available for production and marketing of this product with CMFRI as knowledge partner.

Prospects of development of a trade through hatchery production

The damaging fishing methods which destroy the fragile corals and over harvesting of the species in demand are the vital problems associated with the trade. It is widely accepted that the ultimate answer to a long term sustainable trade of marine ornamental trade can be achieved only through the development of hatchery produc-

tion technologies. In this context it is imperative to develop commercially viable seed production techniques of species which are in demand. It is well accepted as an environmentally sound way to increase the supply of marine ornamentals by reducing the pressure on wild population and producing juvenile and market sized fish of wide

variety of fish year round. In addition hatchery produced fish are hardier and fair better in captivity and survive longer. The methodologies developed by CMFRI can be scaled up for commercial level production and a hatchery produced marine ornamental fish trade could be developed.

Bivalve mariculture in India – progress in research and development

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Introduction

We are all aware that with an average growth rate of 6.9% per annum, aquaculture is the fastest growing food production sector in the world (FAO, 2009) and now accounts for nearly half of the global fish production. Given the projected population growth over the next two decades, it is estimated that at least an additional 40 million tonnes of aquatic food will be required by 2030 to maintain the current per capita consumption. Among the Asian countries, India ranks second in aquaculture and third in capture fisheries production and is one of the leading nations in marine products export. In mariculture, India has been a late starter in developing and commercializing technologies. I recall that in the nineteen eighties, as Director of CMFRI, I gave impetus to research programmes on culture of a number of marine species, such as, mussels, oysters, pearl oysters, sea cucumbers, seaweeds and the like which has now led to a fledging small-scale industry. Until then, mariculture was confined to traditional shrimp culture practices. During the nineteen eighties and nineties several comprehensive technologies, including hatchery techniques for production of seeds were developed for farming in coastal water bodies.

Traditionally, in India, bivalves have always been considered as subsistence food of the poor, save for pockets of high consumption like the Malabar and Goa coasts. In Malabar and Goa culinary preparations with bivalves go a long way back in history. But, taking into account the status of bivalves in international aquaculture production (a third of the total by weight) and trade, focus was placed on developing technologies for its farming and hatchery production of seeds. Through this focused attention, techniques for farming mussels, oysters and pearl oysters were developed by the CMFRI in the nineteen eighties, but they did not achieve commercial status until the mid-nineties. In the case of mussels and oysters due to concerted technology transfer efforts by CMFRI from the nineties, the combined production has crossed 20,000

tonnes making India one of the top-ten countries in Asia in bivalve mariculture production. In marine pearl culture too India has made significant achievements in developing a pearl production technology, besides a protocol for hatchery production of pearl spats. However, several issues hinder its development on a commercial scale. Let us examine in brief the progress in farming for each of these commodities.

Mussel farming

Although the technology for mussel farming has been demonstrated in several locations within Kerala State and in different maritime States, the diffusion of the technology was predominantly in northern districts of Kerala and now rapidly spreading to other southern districts as well. Several reasons, such as, fast growth of mussels because of favorable hydrological and geoclimatic conditions, availability of seed from nearby coastal areas, and availability of loans and subsidies from banks and development agencies have been identified as contributory factors for this development. Three types of farm ownerships are observed: individual, family, and ownerships by self-help groups (SHGs). The adoption curves are such that there were only a few adopters initially followed by an increasing rate of adoption in the subsequent years because of the demonstration effect. There is a deep-rooted “risk aversion” attitude widely prevalent among technology adopters. Age could not be significantly related to technology adoption, while education and occupation of the respondents significantly influenced the technology adoption process. The biggest outcome of mussel farming in Kerala was the empowerment of women with 87% of the SHG farms owned by women. The successful diffusion of mussel farming is the result of a combination of factors, chiefly, the availability of suitable water bodies; high rate of education; proximity of mussel markets and high degree of mussel consumption in the area; and a unique synergy between technology developers (CMFRI), promoters (State development agencies such as BFFDA and ADAK), and credit advanc-

ers (local cooperative banks). This development scenario can surely work as a role model for other states and developing nations where similar hydrological, social, and market environment exists.

The basic method of farming developed and promoted by CMFRI in India is by constructing trestles (called racks in India) and suspending the seeded ropes from the horizontal platform in shallow seas and estuaries, though in certain regions, seed mussels are just sown on the substrate (on-bottom) and farmed.

The annual production of farmed mussels has shown a gradual increase from 1997 and it was steep particularly from 2003. On-bottom farming, which is a custom of simply relaying of seed mussels with low inputs, contributed 19% to the production. In certain estuaries, where the depth is less than 1.5 m, the seeded ropes are not hung vertically; rather, they are tied horizontally parallel to estuary bottom. The value of the mussel produced is estimated at \$US 12 million on the basis of farm-gate prices during the period 2009–2010. The total area utilized for trestle farming in 2005–2006 was estimated at 14.14 ha, and on-bottom farming was done in 11.17 ha in the state mainly at Kozhikode and Malappuram districts. The average productivity for trestle method was estimated at 564.9 tonnes/ha, while for on-bottom method, it was 171.9 tonnes/ha. However, there were regional differences in productivity, with high values in Kasaragod and Ernakulam and comparatively low values in Kozhikode and Malappuram.

Credit constraints can be a problem for small aquaculture farms in developing countries and it can actually impede adoption. The subsidies provided by the government agencies served to attract villagers to the mussel farming technology, and these first-time adopters continued the farming activity even after cessation of the subsidy after the first year. Obviously, it is the profitability and creditworthiness of mussel farming technology that has driven the adoption process in Kerala. The rate of returns from mussel farming ranged from 190 to 350% and the capital recovery factor ranged from 1.7 to 3.3, depending on the location. As a spin-off, several small business enterprises which supply other inputs for farming have also been established; the economic value of which has been assessed as nearly a million dollars.

Refinements in mussel farming technology have been made by CMFRI to reduce capital costs (mainly on nylon ropes) by using alternate core materials and pre-stitched cotton net tubes. Seeding is one of the most critical activities in mussel farming. The process which is physically demanding (as farmers have to kneel and bend down to do it) is crucial to the success of farming as the uniform attachment of mussel seed around the rope is dependent on how well it is done. Now, to reduce the physical strain and to increase efficiency during this process, a semi-automated mussel seeder has been designed, developed and field tested. Both old and new farmers have adopted this technical advancement. The chief advantages of the seeder are reduction in time taken for seeding resulting in increased efficiency and lower labour costs and reduction in physical strain during the process. The time taken for manual stitching of 1m rope by the conventional method is 8 minutes whereas in the seeder the same can be accomplished in 2 minutes.

Another innovation to easily separate the mussels from the rope during harvest a semi-automated mussel declumping machine has also been developed. The machine had two separate units, a metal drum and a metallic circular fixed shield with a central opening with a diameter of 10mm fixed on a stand and a ramp for placing the harvested rope. One meter mussel rope could be de-clumped in two minutes. The chief advantages were that physical exertion during harvesting could be avoided and that it was more hygienic and efficient.

Oyster farming

Growth in commercial oyster farming in India has not been as phenomenal as that of mussels. Again the state of Kerala, particularly the southern districts, has taken the lead. Although, oysters form an integral part of the biota in intertidal areas all along the Indian coast, oyster fishing is mostly at a subsistence level catering to very restricted local markets, particularly in the states of Kerala, Maharashtra and Goa. The oyster farming technology developed by CMFRI in the nineteen seventies following the rack and ren and rack and tray methods could not be commercialized for more than 20 years due to lack of consumer demand. Again, it is the concerted technology transfer efforts by scientists of CMFRI that has led to a

commercial practice. The technology adoption has been slow, mainly because of the difficulty in post harvest handling of oysters and the limited markets. Even among oyster consumers, the preference is for cooked meat, rather than whole and live, making heat shucking a necessity. Heat shucking is tedious in the case of oysters as compared to mussels, as they open their valves only on strong steaming. Besides, oyster processors invariably complain about cuts and bruises on their hands while shucking the oyster meat. So much so, many first-time oyster farmers in Ashtamudi, Kayamkulam and Vembanad Lakes of Kerala have switched to mussel farming. However, this trend is recently being reversed due to better market price and also the realization that oysters are more euryhaline than mussels, and hence more conducive for culture in an estuarine environment.

Women SHGs are in the forefront of oyster farming activities, with nearly 2000 families from central and south Kerala being involved. Production has touched nearly 2500 tonnes, and oyster farming has developed as a small-scale industry. Activities related to seed collection, seeding, heat shucking and marketing has led to economic empowerment of villagers especially women.

The development of hatchery technology for oyster seed production paved the way for the expansion of oyster culture into new cultivable areas where no natural stocks were available or natural spatfall was poor. Initially the set larvae (spat) on cultch were transported from hatchery to culture site. Now scientists of CMFRI have been able to develop a remote setting method by which eyed or pediveliger larvae are transported without water, in moist condition to distant places where they are set on the cultch material. The use of this technique has revolutionized oyster farming along the west coast of USA and is expected to make similar impact here too.

The CMFRI has recently taken up an ambitious R&D programme funded by the World Bank to speed up technology adoption in oyster farming in the states of Kerala, Goa and Maharashtra. Through a value-chain approach, it is planned to develop depuration units, value-added products units and an oyster hatchery along the west coast ensuring supply of spats through the remote setting technique. Of interest is the recent attention in live oyster consumption in high-end restau-

rants in metropolitan cities linked to the backwater tourism industry. Initial results indicate that the unit price of oysters can go up by as much as 10-times through this value-chain and can function as a means of attracting new farmers and increasing production.

Impact assessment of farming

Bivalve farming is not an entirely eco-friendly practice of aquaculture as previously thought. Several studies abroad and in India have shown that continued farming in one location leads to bio-deposition and change in benthic in-faunal community structure. Indian farmers are advised not to keep farm location in one place for more than 2 years. The ecological disaster which portends widespread farming of bivalves in semi-enclosed water bodies has also been addressed by scientists of CMFRI. The carrying capacities of some of the water bodies for bivalve farming have been determined and this information needs to become an essential input of the regulatory mechanisms. Farm structures and bivalves obstructs the free flow of water currents through the farm site thereby aiding sedimentation and organic enrichment but the short-term farming period during the impacts were not significant. However, short-term oyster/ mussel farming does not alter the sediment characteristics under the farm.

Mussel watch

Bivalves have been used as sentinel organisms for monitoring contaminants in the marine environment and mussel watch data has been used for assuring seafood safety. They are efficient bioaccumulators of heavy metals, polycyclic aromatic hydrocarbons and other organic compounds, and because they are sessile they may reflect local contaminant concentrations more accurately than mobile crustaceans and finfish species. A recent study indicates that coastal waters of Karnataka and Kerala are minimally contaminated with genotoxic and carcinogenic chemicals.

Bivalves- Organic by default

Organic farming is based on holistic production management systems which promote and enhance ecosystem health, including biodiversity, biological cycles and biological activity. Bivalve shellfish aquaculture meets each of these criteria, and in fact, is probably organic by default. Bivalve

molluscs are not fed so there are no nutrients being added to the marine environment. They are biofilters which feed on phytoplankton which occurs naturally in the water. This biofiltering activity has the beneficial secondary effect of taking up nutrients and purifying the water column, thereby enhancing ecosystem health. Bivalves also create habitat for other marine creatures. As three-dimensional structures, bivalves are host to flora and fauna which make their homes in shellfish beds. These beds also provide cover and forage for fish during their juvenile out-migration stage, enhancing biodiversity, biological cycles and biological activities through the creation of critical habitat. With a view to meet the demands of the discerning customers, and also to enhance the value of the product (by as much as 30%), organic bivalve farming protocols and guidelines have been developed as part of the NPOP (National Programme on Organic Protocols) in India. The focus here has been on classification of bivalve growing water bodies following the regulations of the European Union (EU Directive 2006/113/EC). Currently Indian bivalves are not exported to Europe, as the produce does not meet the monitoring protocols set by the EU. Efforts to meet the regulations are being jointly addressed by the MPEDA, CMFRI, CIFT and EIC and it is expected that exports to the EU would be possible in a couple of years.

Pearl Farming

As a technology very close to my heart, considering that we are coaxing the oyster to produce one of the most bewitching of natural gems, I kept this for the last. The allure of the pearl, the most ancient and most precious of gems is timeless and universal for humans. The pearl has a history more ancient, more fascinating and more regal than any other gem and India has a wealth of marine pearl producing oysters: the *Pinctada fucata* distributed in the Gulf of Mannar, Palk Bay and Gulf of Kutch and the blacklip pearl oyster, *P. margaritifera* in the Andaman and Nicobar Islands. The technology for pearl production, based principally on the Japanese methodology of pearl production, was tried and developed successfully in the Indian pearl oysters mainly through the efforts of Dr. K. Alagarwami and his team of scientists from the CMFRI. Later, in the eighties, they went on to standardize the hatchery protocols for this species too.

Once again, this is a technology developed in the nineteen seventies, but unlike mussels and oysters, yet to become a full-fledged commercial practice in the country. I am glad to understand that through funding from the Ministry of Earth Sciences (MoES), the CMFRI is very seriously attempting to transfer the technology through women SHGs in coastal villages in the Gulf of Mannar, Kerala and Lakshadweep. The newly developed technique of mabe pearl production, which is relatively less skill-demanding, and with fast turnover rates (2 months), serves to attract farmers to pearl farming. The MoES is also funding a project on black pearl production in the Andaman and Nicobar Islands being executed by CMFRI. During the last 7 seven years, this project has been able to establish pearl farms and on-farm grow-out techniques; establish a black pearl hatchery and achieve success in production of pearl spat; develop and standardize mabe pearl production technique and develop technique for continuous mabe production without sacrificing oysters and conduct training programmes to shellcraft artisans and women fishers on mabe production. Black pearl production itself has not been achieved yet, but as I understand, a lot of effort is being put to achieve it. Indeed, the scenario in Indian pearl farming appears poised for a big leap forward and I look forward to seeing it during my lifetime.

Clam Farming

A number of clam species, mainly belonging to Veneridae, Arcidae and Corbuculidae family are fished from coastal waters of India, and annual estimates of catches are close to a 100,000 tonnes. Because of the high inter-annual variability of the resource, many fishers have resorted to re-laying of seed clams in water bodies close to their homesteads particularly in Kerala and Karnataka. Out of the total production nearly 10% is obtained through this semi-culture practice. Earlier, the CMFRI had brought out a culture technology package using pen enclosures for the blood clam *Anadara granosa* with a production potential of 40 t/ha/6 months, however, this has not reached commercial application yet. Currently, major attempts are being made to develop on-bottom and off-bottom clam farming techniques for the black clam (*Villorita cyprinoides*) and the short-neck clam (*Paphia malabarica*), which have reasonably good price structure locally and abroad.

On Gender and Bivalves

The development scenario scripted by bivalve farmers in Kerala shows that women were the major players with more than 4,000 women becoming owners of bivalve farms. Support from the government prompted women to form self-help groups. This led to group farming, which helped women overcome social inhibitions and prove their competence. The fact that women increased the farm area and intensity of farming shows that they became efficient aqua-planners and aqua-managers and it also proved that women are better carriers of development. Their prompt repayment of loans increased the faith of the bankers and the schemes of helping groups continued over the years. Women were therefore all-round players, right from planning to utilization of profit.

Application of Biotechnology

In oyster and mussel farming knowledge of the time of spatfall is very important for farmers to decide on the time for setting spat collectors. This is particularly important when the current farming practice is wholly dependent on natural spat as seed. Through a project funded by the DBT, the CMFRI has achieved preliminary success in developing a PCR based protocol for identification of mussel and oyster larvae from a cocktail mix of various holo and mero plankters (as found in a plankton collection). So far, bivalve farming has not been affected by any serious diseases. Very recently scientists from CMFRI were able to detect an OIE listed protozoan pathogen *Perkinsus olseni* in farmed and wild pearl oyster *P. fucata* from the Gulf of Mannar. A PCR kit for its detection was also developed. It is possible that perkinsosis could be one of the major reasons for the decline of the *P. fucata* beds in the Gulf of Mannar over a period of time.

A recent advancement is the development of a neutraceutical from Indian green mussels, again by scientists of CMFRI, called GME (green mussel extract) which has been found to have definitive anti-arthritis properties mimicking the pain killer drug aspirin. This drug which is now undergoing field trials, is surely a means of value addition to mussels, and bound to improve incomes of mussel farmers.

Prospects for Future Development

It is quite clear from the fast pace of its development in the state of Kerala that bivalve farming

can develop as a new sunrise mariculture industry in India. Unlike other aquaculture industries, it is not capital intensive and offers great scope for improving the incomes of the rural fishers as an alternate livelihood. But primarily, what has spurred its growth in Kerala is the considerable demand for the produce among the populace. Other bivalve consuming states like Karnataka, Goa and Maharashtra can also be targeted in the next phase of development. Policy makers and planners need to address the following for sustained development of this spanking industry.

Mussels & oysters

Promote bivalve farming, particularly mussels, in all maritime states using Kerala as a developmental model.

Since farming depends on seed availability from natural sources, development of methods to collect seeds from the wild is necessary.

Determine carrying capacity of backwaters/estuaries for bivalve farming and restrict farming accordingly.

Make a prospective (5 years) plan to improve hygiene in farming areas using international guidelines as a criterion.

Conduct awareness campaigns for improving bivalve consumption in India

Pearl oysters

Demarcate areas for mariculture and create mariculture zones with adequate legal protection and articulate open-access water body leasing policies.

Promote SHGs to take up pearl farming in identified pearl mariculture zones

Undertake stock enhancement of blacklip pearl oysters in A&N Islands using the hatchery technology developed recently

Priority in research for production of large fucata pearls and black pearls

Processing and marketing

Encourage value added products (VAP) for bivalves to increase marketing possibilities (especially live oysters) and to make the farming practice more remunerative.

Research focus to be placed on pearl processing for improving value.

Mussel farming and hatchery

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Introduction

The world mussel production (FAO data) during 2010 was 1.81 million tons valued at 1.572 billion US dollars. The world production of *Perna viridis* during 2011 was 1,21,322 tons valued at 44.95 million US dollars. The total aquaculture production of green mussels in India (2008) was about 17,000 tons. The farming practice of bivalve molluscs is either on bottom or off bottom culture methods. The bottom culture system is also called the broadcast technique. For the off-bottom culture system, this includes the stake or pole method, rack, raft and long-line method. The rack, raft and long-line method are also called the hanging or suspended culture technique. The stake and rack method are mainly used in shallow, intertidal waters while the raft and long-line methods are generally utilized in deeper, open waters. The culture method followed in the different parts of the world is described.

Many culture techniques are used for growing mussels worldwide and the most popular are described below:

1. Bouchot or Intertidal Pole Culture

In France, mussel culture is believed to have started in 1235, when an Irish sailor Patrick Walton survived a shipwreck on the Bay of Aiguillon. He found that the wooden poles and nets that he had kept for trapping birds attracted mussel spat settlement. This became the basis for Bouchot method which is the oldest and the main method utilised in France on the Atlantic and English Channel coasts. This method, well suited to the large intertidal mud flats facilitated the development of the blue mussel (*Mytilus edulis*) industry in France (Gosling, 2007). The Bouchot method extended to other suitable intertidal areas along the Brittany and Normandy coast. The spats are collected on spat collecting ropes made of coir. These spat bouchots are situated offshore and consists of parallel rows of poles with horizontal coir ropes for collecting seeds. When the seed are a few months old, they are removed from the

ropes, placed in mesh tubes and transferred to bouchots for growth.

Mussel seeds are harvested from August – December depending on the size and density of settlement. The seeds are scraped from the poles using a steel blade attached to a metal wire to hold the scrapped off mussel. In this method, ropes with spat attached are wound around large vertical poles (bouchots) in the intertidal zone. The line of poles mainly oak tree trunks 4-7 m long, 12-25 cm diameter at the wider end and about 7cm at the opposite end. The lower 3 meter of the pole is inserted in the seabed. Mesh netting is used to cover the mussels to prevent them being detached and lost. A barrier is placed at the bottom of the pole to prevent predators such as crabs from reaching the mussels. Bouchot are placed perpendicular to the shoreline and consists of 125 poles running for 50-60m and spaced 15-25m from the next line. This method of culture requires large tidal ranges, in order to supply the densely packed mussels with plankton.

Marketable mussels of 4-5cm shell length are harvested when they are 12-18months old. On an average, 25 kg of mussels are harvested from each pole annually. The entire produce is sold domestically. 4000 poles can produce 100 tonnes of mussel per year.

2. Stake culture

In Thailand and Philippines, mussels are grown on bamboo poles (6-8m long) staked at half meter depth and one meter apart or in circle and tied at the top to form a wigwam structure in soft, muddy bottoms. Mussels (*Perna viridis*) settle on the submerged bamboo stakes. Bamboo poles are often observed to monitor growth as to eliminate predators like starfish and crabs. Bamboo stakes are placed in areas where natural spatfall is expected. Mussels are harvested after a growing period of 6–10 months after stocking or when the animals reach 5–6 cm in length. Each pole yields 8-12 kg of mussel. Harvesting is done by hauling up the bamboo poles and loading them

into a raft. Divers are employed to pick out the larger mussels and the small ones left for the next harvest season. This selective harvesting results in two or more yields within the 6–8 months of the farming period. Harvested mussels are cleaned and then placed in baskets and shaken vigorously in seawater until they are clean of barnacles and dirt. Bamboo poles that are worn out are removed while the good ones are cleaned for the next culture season. The stake method is an economical and easy way of growing mussels but has also some shortcomings. The bamboos decay easily and it is at times difficult to match staking operations with spatfall. This culture system also facilitates siltation which makes bays and estuaries too shallow for mussel farming. In Philippines a rope strung in a zigzag fashion or rope web method is used. Each unit consists of two bamboo poles 5 meters apart are driven into the substratum. Two polypropylene rope, 2 meter apart are tied to the bamboo poles. 40 m rope of 10-12 mm diameter is used to connect in a zigzag manner. Pegs are inserted at 40 cm intervals (Joseph, 1998).

3. On-bottom culture

This method is widely used in Netherlands, Denmark and Germany. The culture is based on the principle of transferring seeds from areas of great abundance where growth is poor to culture plots in lower density to obtain better growth and fattening of the mussel. The culture plots must have a firm substratum and less of drifting sand and silt particles. In Netherlands, the seeds are dredged from Waddenzee. The seeds are laid in intertidal areas to produce mussels with thick shells and strong adductor muscle. In the subtidal areas higher meat yield and thinner shells are produced fit for processing industry. The whole process is highly mechanized from collection of seeds to harvesting and marketing. Waddenzee and Zeeland are the important areas for mussel (*M. edulis*) farming. In Zeeland, the town of Yerseke is the important mussel trading area. Waddenzee in the northern part of Netherlands was used as a source of seed. Since 1950, farming plots were also created here. The seeds which are fished from the seed beds during the short well defined period are scattered evenly on the beds allotted by the government to mussel farmers. The seeds are gathered by special mussel boats. About 10 tonnes of mussel seed can be gathered in one hour of dredging operation. The seeds

gathered are replanted the same day over the plot measuring 500 x 200 meters. 20 to 35 tonnes of seed are used per hectare for relaying depending upon the size of mussel seed. The mussels are distributed evenly by the farmers if the stocking is found crowded. The starfish problem is managed by salt treatment or removal using starfish nets. The filtering activity of the mussels produces silt which gets deposited under the mussel carpet. This hinders the growth of mussels. Chain harrow are used to level the ground. In the Waddenzee, the mussels are usually kept in the same area but in Zeeland the half grown mussels are relocated to deeper areas where conditions for fattening and growth are better. Waddenzee mussel being slightly larger are suitable for half shell trade and Zeeland mussels are preferred as raw material for canning factories fetching higher price. The mussels are marketable in the Dutch mussel farming areas when they are 2-3 years old. The production by on bottom culture is about 8Kg per m² of mussel plot or 80 tonnes per hectare. An essential part of the on bottom Dutch mussel farming is the 'rewatering' process. Here before marketing the mussels, they are kept in special lots for 10-14 days for the process of eliminating the weak and damaged mussel.

4. Long line culture

This method is becoming very successful in open sea mussel farming. A rope is stretched horizontally near the water surface and maintained 1-2 m from the surface with buoys. Mussels are grown on vertical ropes known as 'droppers' which hang from the horizontal rope for a length of 4m. Mussel seeds are collected from natural beds and transplanted onto the ropes into a continuous sock-like cotton tube, which is approximately 17.5 cm in width. Small mussels stripped from the collection ropes are inserted. This cotton sock is then wound around the dropper. The mussels grow and attach to the ropes using their byssal threads and the cotton sock slowly disintegrates and falls away. The droppers are placed a minimum of 0.5 m apart and have at least 4 m of free space from the bottom. In deeper waters the gap between the bottom of the line and the sea floor is greater. Anchor ropes extend from each end of the horizontal rope to anchors buried in the mud of the bottom. As the ropes are kept taut, there is no movement around the anchor to disturb the bottom as occurs when boats are anchored. The

density at which mussels can be cultured on long lines could be about 300 per meter, but depends on the food availability, which varies from site to site. Mussels grown on longlines can become smothered by naturally settling juvenile mussels and other fouling organisms. For this reason, most farmers prefer to position their farms away from heavy spat settlement areas to avoid layers of spat attaching to larger mussels.

5. Raft Culture

The basic principle of raft culture is similar to long line culture in that the mussels are suspended on droppers but these are suspended from the raft instead of the long lines. The raft itself is anchored to the seabed removing the need for several anchoring systems. Long line culture however, creates less of a visual impact, and the droppers can be spaced farther apart to maximize the use of the available phytoplankton. Raft culture is more suited to areas of dense phytoplankton and to smaller operations, as there is less scope for mechanical harvesting. This method of culture is used in the Galician Bays in Spain, Saldahna Bay in South Africa but has been abandoned by the New Zealand industry in favour of long lines. This method has its origin in Spain in the Galician Bay. Mussel seeds (*Mytilus galloprovincialis*) settle profusely in the inter-tidal zone in the coastal waters of Galicia. Rias are deep sunken river valleys upto 25 km in length, 2-25 km wide and 40-60m deep. As these rias are protected by islands at their mouth, these sheltered, nutrient rich rias with 3-4 m of tidal range provide ideal environment for suspended mussel culture. The rafts are constructed using a wooden framework of timber and floats of concrete, steel, styrofoam or fiberglass material. The average size of the raft is 23×23 meter which supports 700 ropes. The rafts are anchored along the sides with large concrete moorings. There are over 3000 rafts in the Galician rias. The rafts are spaced at a distance of 80-100m from each other and positioned in groups called parks. These seeds are collected by scraping the rocks with spade-like steel blades. Seeds can be collected by suspending ropes vertically from the rafts in December and January to catch seeds in February and March. Seeds also settle on the mussel ropes. The length of the mussel ropes varies from 6-9 meters according to the depth of the culture site. Pegs are used at 40 cm intervals to avoid slippage. The average weight of seed per

meter of rope is 1.5 to 1.7 kg. About 4600 t of seed per year are needed to maintain the present level of production (Gosling, 2002).

Thinning out of the mussel ropes are done in 3 to 6 months depending upon the growth. The ropes are removed when the weight attain 10 Kg of mussel per meter. The ropes are hoisted and the mussel transferred to new ropes. About 3.5 Kg of half grown mussels are attached per meter of rope. After 8 to 12 months of growth the mussels attain marketable size of 8-10 cm (Korringa, 1976). Growth of mussels on inshore rafts is less than on rafts in the mouth of the Rias, which demonstrates food constraint at inshore sites (Navarro et al., 1991). Temperature plays an important part on the growth as the mussels in the upper part of water column, above the thermocline (2.5m) were significantly larger than those cultivated in deeper waters (7.5m) (Gosling, 2002). Harvesting is done using mussel boats outfitted with power crane and metal baskets to collect the mussel ropes. As the production is about 10 Kg of mussel per meter of rope, a raft having 600 to 1000 ropes of 6-9 meter may produce 30000 to 90000 Kg of mussel per year. After harvesting, the mussels are kept for depuration for 24-48 hours before they are marketed (Korringa, 1976).

6. Rack culture

This is the simplest of the rope method used for green mussel cultivation in India and Philippines. The main purpose of the pole is to support the structure. In between these poles, ropes are suspended either vertically or kept horizontally where the depth is a limitation. The construction is labour intensive but the simplicity in harvesting and accessibility of local materials for farming purposes makes it very adaptable under local conditions. Mussel culture is fast becoming popular in the Malabar area since 1997 following the success achieved by CMFRI in rearing green mussel by rack culture in the backwaters. The simple methods employed for mussel farming was transferred to progressive farmers who took up mussel culture in the backwaters. Soon they found the venture profitable. Demands came from new entrepreneurs for training and mussel farming spread from Kasaragod to Ponnani. Mussel culture in the backwaters of Kerala was first started in Padanna and Cheruvattur Panchayats in Hosdurg Taluk of Kasaragod district. Later it was

taken to Elathur in Calicut district and Vallikunnu and Ponnani in Malappuram district. The total production in 2008 was 16,500 tonnes. Some of the constraints are regarding the availability of seed. The seeds required for culture is presently collected from traditional fishing areas and these are often causing conflicts between farmers and mussel fishermen. Hence it is essential that additional spat collectors have to be established along the coast to ensure supply of seeds to the farmers.

The harvesting seasons of cultured mussels is mostly during April – May months and farmers are forced to sell their crop before the onset of monsoon to avoid mass mortality of mussels due to freshwater influx into the backwater system. At present only a few processing plants purchases cultured mussels from the farmers and as a result the local market are flooded with cultured mussels during these months resulting in fall in the prices and thereby affecting the profitability of the operation. Siltation in the backwaters is another problem. This often results in mortality of mussels in the farms. Hence scientific feasibility studies are required to demarcate potential culture sites. Mussel farming is a decade and half old farming practice in India. This is a low investment activity with very good returns. If promoted properly, mussel farming can be used as a tool for women empowerment in the coastal areas and can stimulate a healthy socio-economic development in the area. Better post harvest technologies can develop attractive value added products. Since very good export markets are available for mussels there is further scope of extending the farming practice to suitable areas.

Depuration

Bivalves are filter feeders in their feeding habit. During this process they accumulate all suspended biological materials including harmful microorganisms. Before the product reaches the market, these materials have to be removed from their gut. The process of such purification is called depuration. Hence, depuration is the process of purification of shellfish in which the animals are placed in disinfected recirculating or running seawater and allowed to actively filter feed. The process leads to elimination of bacteria from the bivalve. Disinfections of circulating seawater can be achieved by use of UV radiation, ozone treatment, irradiation etc. Simple depura-

tion can be achieved by starving the bivalves in clean and filtered seawater/ brackish water for a certain period of time. More effective depuration can be achieved by using disinfected water in the depuration process.

Depuration process

- a. The basic principle for controlled purification or depuration of bivalve involves providing clean and purified seawater in tanks, whereby the bivalve filter and pump such water for a period of 24 hours or more if required.
- b. Ideally a depuration plant should be located near the least polluted source of water in the vicinity of bivalve farms. Also the physical characteristics (salinity, temperature, dissolved oxygen etc.) of the seawater used in the depuration plant should not be radically different from that of the bivalve farming areas. Care should be taken such that the level of dissolved oxygen should not be allowed to drop below 2 mg/l.
- c. Two concrete seawater storage tanks of the dimension 20 x 8 x 8 m (total capacity 160 tonnes) should be constructed at a level above that of the depuration tank to facilitate gravity flow into the depuration tank (see figure). The water to be used will be first pumped into a rapid sand filter (preferably 2, arranged serially) to remove all suspended material.
- d. The choices for disinfection of seawater are chlorination, ozonation and UV light irradiation. The latter two are expensive, and hence chlorination (@ 3 ppm) is the method chosen for this project. After chlorinating for 12 h, the water will be dechlorinated using vigorous aeration and / or neutralization with Sodium thiosulphate for 12 h.
- e. Most depuration plants use flow through, once through or fill and draw principles. It is proposed here to use the batch process (fill and draw), wherein seawater is drawn from the supply treated with predetermined amount of disinfectant to reduce bacterial levels, stored for a time, then pumped to the tank containing bivalves. The process will be repeated once to ensure complete depuration (see flow chart).

- f. Each depuration unit will consist of one concrete tanks of the size 15 x 4 x 1 m with a gradient of 3% to hold bivalves (see figure). Bivalves will be placed in perforated plastic trays of standard size. The trays in a single tier will be raised from the tank bottom with the help of PVC pipe runners. The tank will have drain plugs at the lower end to facilitate cleaning and flushing.

Run duration and Capacity

- a. The duration of the run will be 24 h, in two cycles with one complete flushing for both mussels and oysters (see flow chart). The unit will have the capacity to hold 1.0 tonnes of mussels and 0.62 tonnes of oysters per run. The water requirement per run will be 144 m³.

Hatchery techniques for green mussel

Mussel farming in India is totally dependent on the wild collection of spat settled on inter-tidal and subtidal rocky patches along the coast. The hatchery produced spats are likely to become important in the future with the scaling-up of commercial mussel farming activities along the coast.

1. Site selection criteria (Sreenivasan, 1998)

Sites adjacent to good quality seawater source is a primary requirement for the setting up of a hatchery. Seawater should be free from pollution, suspended organic and inorganic particles. Water intake near effluent discharge points of industries, sewage treatment plants and river mouths are to be avoided. Salinity range of seawater between 30-35 psu is considered as optimum for year-round spat production. Sourcing of seawater from open coastal areas allows uninterrupted supply of fully saline water with minimum seasonal variations. Sites accessible by road, near mussel farming sites have added advantages. Information on the physico-chemical parameters of the proposed site need to be collected prior to deciding on the adequacy of a site for a hatchery. Remedial measures to improve inadequate quality seawater can be extremely costly and may adversely affect the profitability of a venture.

2. Hatchery facilities

The design and layout of hatchery varies from

site to site, geographic location, level of sophistication, availability of capital and target of production. Hatcheries may or may not include a nursery component. There are two basic parts to a bivalve hatchery, the sea water system and the physical building. An ideal hatchery for producing about five million spat per annum requires not less than 20x10m of built-up area. The hatchery building should be designed and constructed in such a way as to get maximum light and air inside the hatchery. Floors should be of concrete and have sufficient drains. It is much better to have fibreglass tanks so they can be easily moved or changed if needed. All surfaces should be painted with a good quality epoxy resin. Before constructing a hatchery, government regulations controlling discharge of effluents should be reviewed and if they exist they must be followed. Large floor drains sunk into the floors of wet areas are essential and should be located conveniently throughout the hatchery. Periodically large volumes of water must be discharged, e.g. when emptying tanks, and the drains must be able to handle such discharges.

- a. Seawater supply system consists of an intake point, a draw well, sedimentation tank, filter bed, a water sump, overhead tank and delivery lines. Seawater pumped directly from the ocean is first passed through sand filters that filter out most particulate material. A well maintained sand filter will remove the major portion of detritus and organisms from the water that may interfere with bivalve larvae. It also eliminates many of the fouling organisms that could settle and grow in pipes in the hatchery. A series of two or more such filters are generally installed and they are regularly back-flushed to avoid clogging of the filter media. Other types of filters may be used depending on personal preference and cost considerations. The filter bed normally consists of river sand at the top, charcoal, pebbles and granite stones at the bottom. The seawater passed through the filter bed is further purified by passing through 15 μ m 10 μ m and 5 μ m cartridge filters and sterilised in UV chamber prior to use in the hatchery tanks. After filtration, all or part of the seawater is pumped to a storage tank that may be made of either concrete or fibreglass. The daily water requirement of the seawater is

around 10,000 l. Capacity of the storage tank is around 20,000 l and that of the overhead tank is 10,000 l. Necessary electric pumps are to be provided for pumping the seawater at various points. Stand-by motors and generator are also required to meet any contingencies.

- b. Aeration: Air circulation to the tanks is being carried out by using air compressors which can be either of piston or rotary vane type. The air is passed through a series of filters to remove oil and moisture and supplied to the hatchery through PVC pipes. Air can be drawn at the required places from these pipes running the entire length of the hatchery at a height of 3 m through the nozzles. The air is supplied to the culture tanks through diffuser stones. Electrical air blowers are also used which can supply oil free air.
- c. Algal culture facility: The success of a bivalve hatchery depends on the production of algae. Large quantities of high quality algae must be available when needed. It is a most important part of any hatchery and considerable thought should be given to providing a sufficient and efficient working area for this purpose. Since algae are used in all phases of production, the facility should be located centrally and conveniently. A small room is required to maintain stock cultures of algae. Flagellates measuring less than 10 μm are the main food of bivalve larvae while mixed algal culture are used for feeding the spat and seed. The important species used in the bivalve culture system are *Isochrysis galbana* and *Chaetoceros calitrans*. Walne's medium is used for the maintenance of stock culture as well as for mass culture. Normal room temperature is not ideal for the maintenance and culture of flagellates. Hence air conditioned rooms are used which have 23-25° C during daytime. One of the most important factors determining the successful culture of the microalgae is the type and quantum of illumination. Too much of light will cause the culture to decline earlier.
- d. Stock culture: Haufkin flasks with Walne's medium are inoculated with the microalgae. The flasks are placed under tube lights (800 lux). When their maximum exponential

phase is reached, light intensity is reduced to 400 lux to enable further growth. Normally the flagellates will enter the stationary phase of growth after 12-15 days. In this phase, the culture can be kept for a period of 2 months without aeration.

- e. Mass culture: Using the inoculum from the stock culture room, the flagellates are grown in large scale in 20 l glass carboys or in 100 l perspex tanks. Fully grown stock culture is used as inoculums for the mass culture.
- f. Broodstock holding and spawning area: Space is required to hold and condition broodstock. The amount of space needed depends in part on the number of species being held and whether some or most of the conditioning will be undertaken in the open environment rather than in the hatchery. Space is required for spawning trays but this can be part of the larval rearing area.
- g. Larval culture area: Another major part of the hatchery is occupied by the larval rearing facility and dimensions of this area depend on the scale of production. The space is occupied with tanks, the number needed depending on production levels and the techniques used to rear larvae. Larval rearing tanks are generally made of fibreglass (1000 l) and should be thoroughly leached prior to use.

3. Seed production technology

- a. Collection of broodstock: Broodstocks required for induced spawning are selected keeping in view of the area, growth, condition factor size and age of the standing population. They are collected from population where they are known to occur in healthy condition. The prevailing environmental conditions of the area has to be taken into consideration, since based on these factors only manipulation of temperature regime is effected –for conditioning the broodstock for maturation and induced spawning.
- b. Conditioning of broodstock: The selected broodstock are cleaned thoroughly and placed on a synthetic twine knit PVC frame in FRP tanks. Filtered seawater is filled in the tank and well aerated. Mixed phytoplankton cultured in outdoor tanks using sterilised seawater, are added twice a day. The brood-

stocks are conditioned about 5°C below the ambient temperature. Periodical examination of the gonads is made to assess the maturity of the gametes. On observing suitable maturity, the brood stocks are transferred to spawning tanks.

- c. Induced spawning: Thermal manipulation by raising the water temperature few degrees above the ambient temperature is found to be effective to induce spawning in most of the bivalves. Chemicals such as Tris, hydrogen peroxide and sodium hydroxide were also found to induce spawning.
 - d. Larval rearing and spat production: Soon after spawning, the adult mussels are removed from the spawning tank. The water in the tank is kept without disturbance for the fertilization to take place. After fertilization, the seawater in the spawning tank containing the fertilized eggs is diluted several times and the eggs were allowed to develop. After 24 hours, the D-shaped larvae are transferred to 1000 l FRP tanks at the rate of 2 larvae/ml of seawater. Feeding with microalgal food is initiated from the first day after spawning. Quantity of algal cells supplied is dependent on the number of larvae and is also increased gradually with growth of the larvae. Water change is undertaken once in two days. Mild aeration is also resorted. Utmost hygienic conditions are maintained in the hatchery with proper cleaning of the containers, sieves, tubes and aeration stones. Since larval growth is influenced by larval density, food supply, water quality, water temperature and other factors, regular monitoring is done on the water quality and conditions of the larvae. Records are maintained on initial larval density, growth and number of spat settled.
 - e. Nursery rearing: Spat settled in the hatchery tanks were transferred to the nursery either in open sea or in enclosed bay systems for further growth. After attaining suitable size for transplantation, they are transferred to the farms.
- b. The 8-celled stage is observed after 40 min.
 - c. Larvae with apical tuft of cilia and long flagellum is attained 6–8 h after fertilization
 - d. 'D'-hinge veliger by 20–22 h. The 'D'-hinge shells of the veliger (Prodissoconch I) are transparent with conspicuous granules. The velum is well developed, with a velar hood covered with small cilia that aid in fast clockwise circular movements of the larvae. The internal organs are heavily granulated. The straight hinge larvae measure 70–90 μm in the anteroposterior axis and 60–70 μm in the dorsoventral axis. The larvae are very active, spinning and swimming around rapidly.
 - e. Umbo stage: The straight hinge stage, transform to umbo stage on day 7. This stage is characterized by yellow digestive caecae, concentric ridges and lack of radial striae, typical of Prodissoconch II. The larvae, range from 90 to 260 μm in the anteroposterior axis and from 70 to 240 μm in the dorsoventral axis. The larvae which were clam shaped with both valves equal become more globular and develop mantle folds.
 - f. Eye spot stage is characterized by the presence of a black rounded spot below the food mass. The eye spot and the rudimentary foot became distinctly visible by days 13–14 and the larvae measure 220–370 μm in the anteroposterior axis and 200–330 μm in the dorsoventral axis. The eye spot became deeply pigmented and ctenidial ridges develop in the larvae.
 - g. Pediveliger stage The development of the functional foot indicated the pediveliger stage by the 16–19th day. The larvae measure 280–400 μm in the anteroposterior axis and 360–380 μm in the dorsoventral axis. Larvae at this stage are capable of swimming with the velar cilia as well as crawling with the foot. The velar crown is reduced in size and the larvae transform from the free swimming pelagic larvae to the creeping, crawling benthic stage ready to attach to the substratum. Gill filaments were clearly visible. The

50 μm in diameter. Fertilization is complete within 20 min of spawning and the first and second polar bodies are observed 20 min after fertilization.

4. Developmental stage of mussel seed

(Laxmilatha, et al., 2011)

- a. Eggs released by the females are brick red in colour, spherical in shape and measure 45–

radial ridges were also distinct and green coloration was noticed along the margins of the shells.

- h. Plantigrade stage: The pediveliger, at the end of the crawling stage settle on the substratum and become plantigrade and begin its sessile life. Spat settlement could be observed from the 21st day onwards. The larva measure 400–490 μm in the anteroposterior axis and 380–480 μm in the dorsoventral axis. The velum disappears, labial palps appear and additional gill filaments appear. The concentric growth lines, foot, heart, posterior adductor muscle, mantle edge, visceral mass, intestine and chromatophore pigments are distinctly seen.
- i. Spat: The plantigrade transform into young spat by developing the characteristic adult shell. The shell by now assumes the typical oblong shape like the adult mussel. The hinge line, the anterior and posterior auricles and the byssal notch typical of the adult mussel are formed. The spat attached to the bottom substratum by secreting the byssus threads. Spatfall or settlement began on the 21st day and continued up to 35th day. The spat measure $510 \times 390 \mu\text{m}$ on 21st day and $910 \times 460 \mu\text{m}$ on 28th day.

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Broodstock and hatchery management techniques of sand lobster *Thenus unimaculatus*

Joe K. Kizhakudan

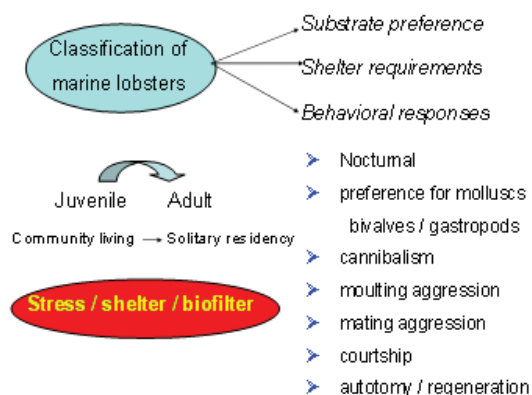
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Introduction

Lobsters are the most valued of all seafood delicacies and lobster tails are always in great demand world-wide. Freshwater crayfishes, which are considered a delicacy in many parts of the world, are a favorite aquaculture candidate in North America, Europe and Australia. Crayfish and rock lobster aquaculture practices are initially capital oriented but deliver high production and income turnover in the long run. This industry has already taken off in countries like the U.S.A. and Australia. Lobster culture in India is still in the infancy stage and C.M.F.R.I. has been spearheading research in the development of culture technologies for different species of lobsters.

The spiny lobster *Panulirus homarus* has been the chief candidate for lobster aquaculture research in India. The sand lobster *Thenus unimaculatus* which contributes to 8% of the global lobster production, and ranks next to spiny lobsters and tiger shrimp in export value, is one of the most promising candidates for lobster aquaculture in India. Increasing demand for live lobsters in the export market led the farmers and entrepreneurs to collect juvenile lobsters and crabs from the wild and grow to marketable size in ponds and tanks by feeding trash fishes and other discards. In some maritime states juvenile lobsters, pueruli of *T. unimaculatus* are grown in captivity. Eyestalk ablated lobsters have been found to attain sizes up to 180 – 200 g in 5 – 6 months period. This type of lobster fattening at a stocking density of 10 – 15 young ones per square meter yielded appreciable growth rates with a profit margin of INR.50,000/- from a pond of 70 m². Complete larval development of *T. unimaculatus* was achieved for the first time in India at the Kovalam Field Laboratory of CMFRI. The larval cycle is completed in 26-30 days and juveniles attain a size of 150 g (the minimum legal size for export) in about 300 days. The relatively shorter duration of the larval phase is an advantage in captive rearing of the sand lobster as compared to the spiny lobsters.

As in any aquaculture system, broodstock development and hatchery management are the primary aspects to be tackled while establishing an aquaculture unit for lobsters. Sub-adult and adult lobsters are usually collected from the wild and acclimatized to captive holding. Different techniques for induced maturation and breeding in captivity involve physical handling and provision of favorable influential factors like artificial and natural diets, shelters and hiding places, pathogen-free rearing medium etc. The life history of lobsters shows a transition for a free-swimming planktonic larval phase to a benthic, crawling adult phase. We need to understand the specific requirements of the species before designing the right type of broodstock and hatchery units. The design of an indoor lobster broodstock and hatchery unit is based on the inherent nature of the animals, as depicted below –



Broodstock management techniques

Juvenile and adult lobsters are primarily benthic forms preferring to crawl along the bottom of the sea where light penetration is minimal. To simulate natural conditions to the extent possible, broodstock tanks are usually painted black on the insides and kept covered with dark screens. Lighting in the broodstock unit is kept minimal. The time of light exposure for each species has to be fixed based on experimental studies. Habitat preferences are marked among lobsters. Sand lobsters

are seen predominantly in sandy substrates and spiny amongst rocks. Broodstock tanks for sand lobsters are provided with a layer of sand at the bottom, in which the lobsters remain buried for a major part of the time. Spiny lobster broodstock tanks require no bottom substratum but need to be provided with structures that provide surfaces or crevices for attachment and sheltering. Water quality and photoperiod were found to play a major role and animals reared in larger tanks with increased water depth show more amenability to captive maturation. Broodstock maintenance and development in sand lobsters are done in a Closed Recirculatory System with fluidized bed filter and minimum light exposure (LD 1:23). Juvenile (<30 mm CL) and sub-adult (30- 40 mm CL) lobsters collected from the wild and reared in recirculatory systems developed into mature adult lobsters (65 - 70 mm CL) in a period of about 6 – 8 months. Regulation of light exposure and feeding @ 5% of body weight in two divided doses daily give good results.

Like all crustaceans growth in lobsters occurs in stages combined with a molt. Molting is controlled by hormones. Growth is faster in the juveniles and slows down as the adult phase progresses. Beyond maturation, growth, particularly in females tends to be slower. Lobsters, like other crustaceans, prepare well in advance to molt and have a short phase of starvation at during and immediately after molting, when they are soft shelled and vulnerable to attack by other lobsters in the broodstock tank. This is particularly seen in the case of spiny lobsters which exhibit tendencies for cannibalism. Therefore, it is necessary to provide shelters and hiding places for these animals in the tank, for seclusion during molting. PVC pipes, asbestos tiles, vertical net screens are some of the commonly used structures for this purpose. Juvenile lobsters coexist in a community living structure while adult lobsters prefer a solitary existence. This also necessitates providing shelters to aid in this transition phase in broodstock development.

Food is a major factor determining the performance of the animals in captivity. Lobsters show a preference for shellfish, particularly mussels. Sand lobsters show good reception to fresh clam meat. Broodstock diets should be combination of natural diet preferred by the species and artificial diets prepared to meet the protein

requirements of the broodstock, with additives to promote growth and maturation.

Collection of lobsters from the wild entails the possibility of the animals harboring pathogenic microbes. Quarantine measures and prophylactic treatments form an integral part of the broodstock management unit. This, combined with a strict regime for seawater treatment and disinfection of tanks between stockings, should be good enough to ensure a healthy environment for the lobsters. One of the major problems seen in lobsters, particularly spiny lobsters, is tail injury caused due to aggressive behavior among themselves. Attacks on soft shelled lobsters also induce injuries which tend to get infected. As mentioned earlier, shelters and crevices are essential to avoid such occurrences.

Hatchery management techniques

The larval phase in mostv lobsters is usually complicated, extended and highly dependent on external factors. Like other crustaceans, lobsters begin life as a developing embryo inside an egg which is carried by the female along with hundreds or thousands of other eggs, on the pleopods. These egg-bearing females are called "ovigerous". Fertilized eggs are dark yellow or orange in color and turn dark brown at the time of hatching. Unfertilized eggs remain cream or pink in colour and are shed off in 3-5 days. After a rigorous incubation phase (early embryo development inside the eggs) when the eggs are fanned with the help of the pleopods, small, transparent, flattened larvae called "phyllosoma" hatch out. The incubation period varies from 26-30 days in tropical spiny lobsters to 30-37 days in sand lobsters. Hatching takes place in batches only during the early morning hours and is usually completed in 1-3 days. Water quality, tank bottom quality and handling stress, particularly during the incubation period, greatly influence the success rate of hatching.

Larvae are usually small when compared to the adult except in clawed lobsters. These larval stages (phyllosoma) undergo progressive molts to complete metamorphosis before settling as the post larval stage, called "puerulus" in spiny lobsters and "nisto" in sand lobsters. The hatchery phase is often the crucial stage in lobster aquaculture, since handling of the delicate phyllosoma

is very difficult, and renders the hatchery phase labour intensive. The number of larval stages varies greatly among species, ranging from about 12 stages in spiny lobsters to 4 stages in sand lobsters. Compared to the spiny lobsters, the hatchery phase is of shorter duration in sand lobsters. While larval metamorphoses can extend up to 300 days in spiny lobsters, it is usually completed in 25-30 days in sand lobsters.

The phyllosoma are mostly phototactic and prefer specific zooplankters as live feed. The rearing system should accommodate only minimum numbers per litre, as most of the species are aggressive and cannibalistic; while 10 phyllosoma per litre in tropical spiny lobsters in the initial stages is fine, as stages progress beyond fourth the density has to be thinned further to 5 and 1-2 per litre towards the final stages. The equivalent stages of most species follow almost the same stocking density limits. Larval rearing tanks are usually of shallow depth with upwelling and flow through designs ensuring very less water agitation and reduced photoperiod intensity. Light source is used to pool the larvae to facilitate collection and shifting. Suitable artificial, preferably gel texture, supplementary diets are essential in lobster hatchery feeding regimes. These diets should be floating and stable in water. Water quality in phyllosoma rearing is of utmost importance as delay in

molting attracts too fouling microbes on the shell which render the larvae immobile and obstruct their feeding activity. Organic load and ammonia load should be minimal in the system and tank surfaces should be devoid of biofilm formation to reduce bacterial invasions. Proper feed and health management can improve larval survival and growth to a great extent.

The success of any aquaculture enterprise depends on the efficiency of the rearing system design and its management. Simulation of conditions as close as possible to the lobster's natural environment must be attempted at every stage of its progress from juvenile to adult in the broodstock unit and from egg to juvenile in the hatchery unit. The first hurdle being the steady supply of brooders, the primary aim of the enterprise should be to turn out a good number of adult lobsters developed from wild collected juveniles, and to induce repeated maturation and breeding in captivity. The next hurdle would then be to effect successive larval metamorphosis with high survival rates and post-larval settlement to produce healthy juveniles which would then be ready for generation of a new batch of brooders. Both these aspects can be achieved through a rigid and structured set of management practices as described in this note, but perfected best through practical handling and knowledge gained through experience.

Seed production and farming of blue swimmer crab *Portunus pelagicus*

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Introduction

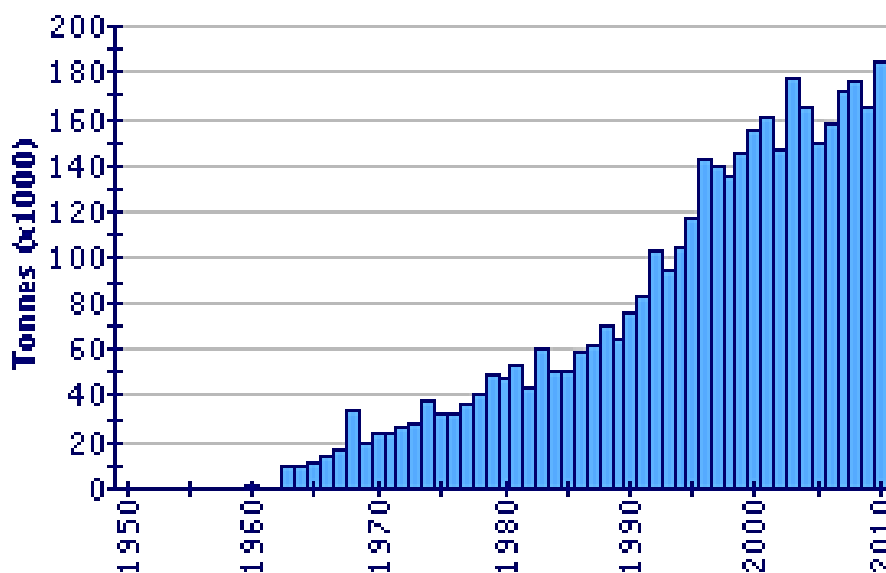
The demand for Blue Swimmer Crab, *Portunus pelagicus* (Linnaeus, 1758) as a delicacy has increased many fold during the recent past; in the fishery, its status as a 'by catch' is changing towards 'target fishing'. Studies conducted in CM-FRI has proved the suitability of the species for mass seed and farming (Josileen, 2001, 2005 and Maheswarudu et. al., 2008).

P. pelagicus is one of the major marine crab species landed in India. Though it is distributed throughout Indian coast, bulk of the landing is from Palk Bay and the Gulf of Mannar region, along the south-eastern coast. Crabs are mainly caught in bottom trawl nets, operated in deeper waters upto 50 metres and in some indigenous gears like modified gill nets which are used ex-

clusively to catch crabs (locally known as Aedi bale, Nandu valai and Peethu valai), and mostly restricted to shallow grounds upto 15 metres. In other countries, several types of fishing gears are used to catch Blue Swimmer Crabs and among them crab pots being more common.

World fisheries for blue swimmer crabs are dominated by three species, *Portunus trituberculatus* (Japanese "gazami") (50%), *P. pelagicus* ("blue swimming crab") (25%) and *Callinectes sapidus* ("blue crab") (25%) (Secor et al., 2002). The market for this species has expanded considerably with the export of processed crab meat into the U.S. The global landing of the species during 1950-2010 is shown in the following graph.

Global catch of *Portunus pelagicus* during 1963-2010 (FAO)



Distribution

Geographical distribution of *P. pelagicus* ranges from Red sea, Mediterranean, East coast of Africa, Persian Gulf, Pakistan, India, Sri Lanka, Mergui Archipelago, Singapore and Philippines to Australia, New Zealand, Tahiti, China and Japan. The Blue Swimmer Crab consisted of one species

until 2010, when new genetic information resulted in the division of this species into four separate species (Lai et al. 2010).

Availability of quality seed in required quantity during a prefixed time is one of the essential requirements for the successful crab culture. Marine crab seed is not easily available from the wild

and if at all available, it should not be collected from the wild for the sake of conservation. A thorough knowledge in biology and larval cycle of the species is very much required for the mass seed production and culture; hence different aspects are briefly described here.

Biology

In *Portunus pelagicus*, sexes can be easily differentiated from their colour patterns of dorsal exoskeleton. Male crabs, are bigger and more colourful than the females, with a dark-blue carapace, pale belly and rich blue on their legs and claws (hence the name, blue swimmer crab). But female crabs are dull brown in colour with small irregular white patches on the carapace and tips of chelate and walking legs are dark brown. Males also have longer claws in proportion to their carapace than females. However the easiest way to check if a blue swimmer crab is male or female is to turn it upside down and look at the shape of the abdominal flap. A male's flap is narrow and angular (inverted "T" shaped), while a female's flap is broad, conical/oval to rounded depending on its maturity stage (Josileen, 2001).



Blue Swimmer Crabs are a fast growing species of crab that can live up to 2.5 - 3 years, weigh up to 1 kg and reach a width of 20 cm depending on its sex and region. Length-weight relationship analysis in *P. pelagicus* shows that in juveniles and pre-adult crabs, weight gain is almost uniform; females are slightly heavier than males until they attain 120-125 mm carapace width. Thereafter males are heavier than females at any given length (Josileen, 2011b).

Male has pleopods modified as copulatory organs on the first and second abdominal somites. In the case of females the first four abdominal somites carry pleopods, and are biramous and possess setae for attachment of the extruded eggs till hatching.

Food and Feeding

Knowledge of the dietary habits of a species is essential for understanding its nutritional requirements and thus useful for its successful culture. The diet of *Portunus pelagicus* was similar in several aspects to the diet of other portunid crabs. Studies conducted in the Palk Bay- Gulf Mannar region, confirmed that they are opportunistic omnivores with a preference for animal food. There are also significant differences in the preference for food items in the different size groups of the crab and *P. pelagicus* exhibits, in this region at least, a clear preference for crustaceans (Josileen, 2011a).

Fecundity

The number of eggs present in the sponge/berry in *P. pelagicus* ranged between 60000 and 1976398. The average number of eggs for the different classes is given in the following Table*.

Size range (mm)	Average tot. no. eggs	Egg mass index
100-109	203455	15.95
110-119	214175	11.39
120-129	640431	16.78
130-139	470092	12.97
140-149	936731	13.51
150-159	1267022	10.56
160-169	1230900	10.78
170-179	1472240	12.24
180-189	1677168	10.03

*(Josileen, 2013)

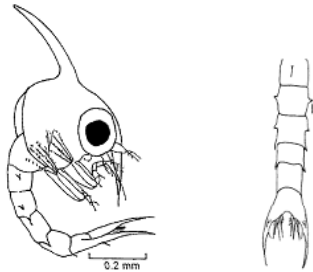
Larval stages

The larval stages included four zoeal stages and a megalopa stage. The megalopa moulted to the first crab instar. The zoeae and megalopa were very similar to those of other portunids. Each Zoea has a long rostrum, a dorsal spine and a pair of short lateral spines on the carapace. The duration of each of the first two zoeal stages was 3-4 days, the following two stages 2-3 days, and the megalopa 3-5 days, reaching the first crab stage in 15-17 days.

First zoea

Carapace length varies from 0.44 to 0.54 mm

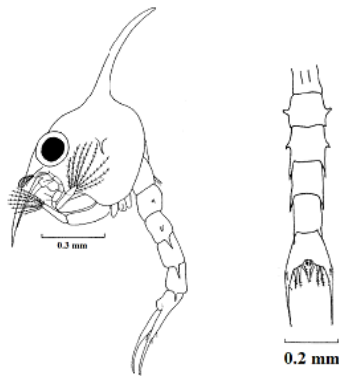
and abdomen – telson length from 1.07 to 1.23 mm. Eyes are sessile. The first abdominal segment bears a short seta on its dorsal surface. Abdomen five segmented plus the telson. Telson forked, with each fork bearing, one inner and one dorsal spine. Inner margin of each fork bears three long and serrated setae.



Zoea-I

Second zoea

Carapace length 0.72-0.77 mm abdomen-telson length 1.46-1.54 mm. Eyes are stalked. Abdomen as in previous stage, except for pair of medium-sized setae on dorsal surface of first somite. Abdominal somites 3-5 have more distinct lateral spines. In telson a pair of short, plumose setae added on median margin of cleft part. Other structures as in previous stage.

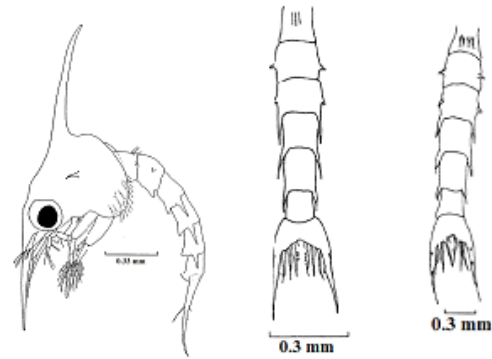


Zoea-II

Third zoea

Carapace length varies from 0.79 to 0.87 mm and abdomen-telson length between 2.02 to 2.21 mm respectively. Dorsal surface of the first abdominal segment has 3 median short setae. Rudimentary buds of the thoracic appendages are developed behind the second maxilliped. Abdomen six segmented; dorsal surface of first abdominal somite has three median short setae. Lateral spines on somites 3-5 longer. Paired pleopod

buds at ventral posterior end of somites 2-5 and telson similar to that of previous stage.



Zoea-III

Zoea-IV

Fourth zoea

Carapace length 0.98 -1.06 mm; abdomen-telson length 2.61-3.03 mm. Pleopodal buds in the abdomen well developed: biramous on somites 2-5, uniramous on somite 6. Dorsal surface of first abdominal somite has four median short setae. Telson similar to that Zoea-III, except for additional short seta on inner margin.

Megalopa

Very similar to that of other portunids. Rostral spine present. Eyes project as far as lateral margin of carapace. Carapace length (including rostrum) 1.69-1.81 mm, width 1.16-1.31 mm. Abdomen six-segmented, with dorso-ventrally flattened telson. Abdominal length (including telson) 1.31-1.35 mm. Total length including rostrum 3.0-3.2 mm.



* For details of larval description refer Josileen and Menon (2004).

Larval rearing and seed production

Collection of broodstock: *Portunus pelagicus* is a continuous breeder, so the berried crabs are available throughout the year. Healthy ovigerous females with characteristic yellow/orange coloured eggs can be collected from sea and brought to the laboratory in jerry can with sea water. These crabs are kept in 1.5 t capacity fiberglass tanks at a salinity of 32–1 ppt, pH 8.2–0.1 and temperature 28–10°C with continuous aeration. Only filtered seawater is used for the entire rearing operation and 50% of water exchange must be daily given. Usually, the berried crabs do not feed and hence feeding is not required.

Broodstock Development in Captivity

Brood stock crabs can be raised in captivity using either juvenile crabs collected from the wild or using reared crablets. produced. Five to ten ton capacity round FRP tanks can be used for the brood stock development. The colour of the tank is preferably black to minimize the algal growth and to provide suitable natural environment to the growing crabs. An in-situ biological filter bed of 5-10 cm height was set on a perforated false bottom erected at about 15 cm height over the entire bottom of the maturation pool. 8-12 numbers of PVC tubes of 1 m height and each with 50 mm dia. are fixed vertically in the peripheral region of the sand bed at equal distances. Water column in the pool above the sand bed must be maintained at 0.5–0.75 m depth, depending on the size and height of the broodstock tank. The crabs above the size of 60mm carapace width (CW) are transferred into the pool. Water recirculation was maintained at the rate of 300% by lifting the filtered seawater from below the sand bed through PVC pipes with a lid to reduce light intensity. Air water lifting system is arranged in the tank through air dispersing stones. Daily 15-20% water exchange is given and once in a week 100% exchange was given. If possible it is better to provide a running water facility of slow speed to ensure the best water quality. Water pH must be maintained at 8.0-8.2 by addition of sodium carbonate whenever necessary. Crabs should be tagged individually by sticking labels on middle of the dorsal carapace. Daily the animals are fed ad libitum with clam meat/ shrimp/ squid meat in the morning and evening hours. Faecal matter and unused feed are siphoned out in the morn-

ing hours before the water exchange. Animals are observed regularly especially the female crabs for spawning and its frequency in each moult cycle. After each moult and sufficient hardening of the exoskeleton of the crab, new stickers are attached to the dorsal side of the carapace.

Water quality was maintained in the ideal range as those factors play important role in successful growth and maturation in captivity. Salinity, temperature, pH, dissolved oxygen, total ammonia and nitrate are monitored and kept optimal regularly. Tank water temperature is maintained between 28-30°C and dissolved oxygen between 5-7 mg/l. In such a maturation system with good management practices, crabs will attain maturity within few weeks / months depending on the initial size of the crab. The male crab attains maturity by its 12th moult and female crab by 14th moult. The average size (CW) of the mature male and female crab was 82.3 ± 1.17 mm and 120.4 ± 2.23 mm respectively (Josileen and Menon, 2005).

The crabs are spawned spontaneously, without using any chemicals, hormone or eye-stalk ablation. The incubation period ranged between 8-10 days mainly depending on the size of the berry and rearing water temperature. The zoeae produced from the captive broodstock are healthy and active like from the wild berried mothers. The duration of the larval cycle also similar to those collected from the wild. For best results it is better to use the mother for a single spawning or at the maximum two.



Hatching of zoeae: The changes in the egg colour must be observed daily and when the egg mass changes to deep grey that particular female crab is transferred into a separate tank with known volume of seawater (around 500 liters) during evening hours. Only one berried mother is intro-

duced in a single hatching tank. The total weight and carapace width are measured. The tank must be cleaned and water exchange should be given till of hatching. Anticipating the hatching during the following night mixed phytoplankton dominated with *Chaetoceros* spp. (10000 cell/ml) and rotifers (5no. /ml) are added in the hatching tank.

Hatching takes place during early morning hours. After full hatching mother crab is removed from the tank and weight of the crab has to be taken. In hatching tank aeration is stopped for few minutes allowing the empty eggshells and un-hatched eggs to settle at the bottom. These are removed carefully without disturbing the live zoeae in the water column and surface. Samples are taken from the tank and zoeae are counted and total zoeal estimate is recorded.

Larval rearing: 1-5 ton capacity round/oval fibre glass tanks are generally used for rearing larvae. Filtered seawater (through 1 mesh filter bag) is used for larval rearing. The newly hatched active zoeae are stocked in the larval rearing tanks at a stocking density of 50,000 no/t. Stocking is normally done during morning hours.

During the entire larval rearing period, every morning 30-40% of the culture tank water is exchanged. During the process tank bottom is cleaned, excess feed and dead larvae must be removed using suitable filter after stopping the aeration. For all the zoeal stages vigorous aeration is

given, while for megalopa stage it is marginally reduced. The desired range of various parameters in LRT's are shown in the following table.

Parameter	Range
Salinity	30 - 33ppt
Temperature	27 - 31°C
PH	8.0 - 8.5
Dissolved oxygen	4 - 8 ml/l
Total ammonia	< 0.1 ppm
Nitrite	< 0.05 ppm

A combination of algae + rotifer can be given for the first zoeal stage. Among the different phytoplankton feeds used *Chaetoceros* found to be the best for the first zoeal stage (Josileen, 2001). For the rest of the zoeal stages a combination of rotifer + *Artemia* and for megalopa, *Moina*/*Artemia* + prawn-egg custard will give the best results. From Zoea- II onwards *Chaetoceros* is not supplied to the larvae. Mortality was recorded throughout the rearing period and mortality was more in the 1st to 2nd stage, 4th to megalopa and megalopa to crab stage.

Feeding Schedule: Based on the results of various mass rearing trials on different larval foods and their combinations, feeding protocol for *Portunus pelagicus* has been standardized. Larval food for different stages and their feeding concentration in the rearing water is given in the table.

Stage	Food	Concentration
Zoea I	<i>Chaetoceros</i> + rotifer	25,000/ml + 40/ml
Zoea II	Rotifer + <i>Artemia</i>	20/ml + 5/ml
Zoea III	<i>Artemia</i>	5-10/ml
Zoea IV	<i>Artemia</i>	5-10/ml
Megalopa	<i>Moina</i> / <i>Artemia</i> + prawn-egg custard	3-5/ml + 20-25mg/l
Crab 1 -3	Prawn-egg custard	20% of the biomass

Harvesting

During the time of baby crab harvest, water in the larval rearing tank is reduced to 1/4th. Then the ball valve is opened gently and baby crabs are collected, transferred to another tank of known volume of water. Based on this, the survival is estimated.

Nursery phase

The baby crabs are stocked either in rectangular, open outdoor tanks (provided with sand bed and additional substrata) or in earthen ponds, at the rate of 400-500/ m². The depth of the water column must be maintained at 80-100cm. For the first week, feeding rate and schedule are followed as in the case of first crab instar. In the second

week of nursery phase, cooked clam meat / small shrimp can be given @ 20% of their body weight /day, in addition to the egg custard. 20% water exchange is given on every alternative days by removing water from the bottom layers. Care is taken to prevent the escape of crabs through the outlet by keeping proper mesh. The baby crabs attain an average size of 10mm carapace width at the end of the nursery phase and are ready to stock in a crab farm.

Farming

Earthen ponds are preferred for the grow-out culture of Blue Swimmer Crab. Pond preparation must be carried out as in shrimp farming to ensure the best environmental conditions for the growth and survival of the growing crabs. For best growth and survival salinity between 25-35 ppt is good. Presently no commercial feed is available in the country for using for grow-out culture of marine crabs. However it can be grown with appropriate sizes of commercial shrimp feeds and rate of feeding can be adjusted using check trays. Sampling for growth must be done once in a fortnight using dragging the bag. About 25-30 crabs for each sampling must be collected, segregated sex-wise and carapace width in mm and weight must be recorded. Within a period of 120 days crabs attain marketable size of 100g size and can be sold in live condition by individually tying them without damaging/breaking their appendages. (For the details refer Maheswarudu et al., 2008).

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Hatchery and farming of spiny lobster

An overview

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Introduction

Spiny lobsters are high value crustacean fishery resource having great demand in the international market, especially as whole live. Caught in trawl, bottom set gill nets and traps, some of the important Palinurid species of spiny lobsters are *Panulirus homarus*, *Panulirus ornatus*, *Panulirus versicolor*, *Panulirus polyphagus*, *Panulirus argus*, *Panulirus cygnus*, *Panulirus japonicus*, *Panulirus echinatus*. In India they form bycatch in trawls along Maharashtra, Gujarat (*P. polyphagus*) in Northwest and Tamil Nadu (*P. homarus*, *P. ornatus*) and Kerala (*P. homarus*) in the southeast and southwest coast respectively.



Panulirus homarus

Panulirus ornatus

The importance of spiny lobsters as an export commodity and foreign exchange earner generated interest in understanding their biology and fisheries aspects. Kittaka and Booth (2000), reported average world catches of spiny lobsters as 77,000 t in the 1990's. They observed that the resource is either over exploited or fully exploited and one of the few ways of expanding production was via aquaculture. Lobster Research in Japan is more than a century old. Here Research into hatchery rearing of Palinurid lobsters began in late 1890's and developed into the 1900's (Kittaka and Booth, 2000). In New Zealand successful larval rearing for *Jasus edwardsii* was completed in 1990's. In India, Radhakrishnan (1977) reared a group of juvenile *P. homarus* (Linnaeus, 1758) to sexual maturity and bred them in laboratory.

Spiny lobsters are good candidate for culture because of - high demand and high value, can be grown in high density, have low protein dietary requirements, good food conversion ratio, disease

resistant in optimal water conditions, can be readily bred and are highly fecund. But the greatest hurdle in culturing them is the lengthy larval life which is amongst the longest for marine invertebrates: *Jasus* sp.-12 to 23 months (Booth, 2006), *P. Cygnus*- 9 to 11 months (Phillips and Melville Smith, 2006); *P. ornatus*- 4 to 7 months (Dennis et al., 2001). *P. ornatus* is considered the best candidate species for aquaculture as they have the shortest oceanic larval development phase (Dennis et al., 2001) and the fastest post larval growth rate, attaining a market size of approximately 1 Kg within 18 months after settlement (Hambrey et al., 2001). In India, *P. ornatus* of 100 g reached 1.5 Kg in 8 months (Radhakrishnan and Vijayakumaran, 2000). Tholasilingam and Rangarajan (1986) reported 12-15 months for *P. homarus* to reach the marketable size (250 g).

In the wild the berried (egg bearing) female migrate to the edge of the continental shelf. The eggs hatch into the larval phase known as phyllosoma. The larvae are transparent, dorso-ventrally flattened and planktonic. They drift in ocean currents during their prolonged development and may travel hundreds of kms (Johnson, 1960). They get recruited to the coastal environments by drifting parallel to coastlines with assistance from wind generated shoreward surface currents. During the day they may concentrate in the vicinity of chlorophyll maximum layer and migrate to surface water at night in particular during periods of new moon. They migrate between 30-60 m depth during daylight (Yeung and McGowan, 1991).

Larval rearing

Japan was first to complete the larval cycle of several species of lobsters. Rearing phyllosoma larvae of *Panulirus cygnus* and *Jasus verreauxi* to settlement was achieved by Australia and New Zealand. Inoue (1978) reared the phyllosoma larvae of *P. japonicus* from egg to final stage in 253 days. The hatchery rearing of the tropical species *P. ornatus* has been accomplished in Australia. The species has rapid growth rate from post lar-

vae to market size and has larval phase of only 5 months. M.G. Kailis in Western Australia produced the world's first hatchery reared *P. ornatus* post larvae in 2006. More post larvae were produced in subsequent years by Lobster Harvest Pvt Ltd, set up for the purpose of commercializing lobster propagation. The larval cycle for some of the palinurid lobsters have been successfully completed: *P. argus*, *P. elephas*, *P. japonicus*, *P. longipes*, *P. ornatus*, *P. pencillatus*, *P. homarus*, *P. interruptus*, *P. polyphagus* and *P. echinatus* are some of the species for which the completion of phyllosoma stages to pueruli is yet to be achieved.

Adult lobsters from wild or juvenile lobsters reared to maturity in captivity are maintained as broodstock. Egg bearing females are also used from the wild for hatchery purpose. The eggs are initially orange in colour and before hatching attains black/ dark brown colour. Incubation period usually is from 15-25 days.



Phyllosoma larva of P. homarus

The phyllosoma larvae of *Panulirus homarus* were successfully reared up to the sixth stage at the Kovalam laboratory (Madras) of CMFRI on an exclusive diet of newly hatched *Artemia salina*.

The larvae moulted through the entire range of stages within a minimum period of 52 days and a maximum of 64 days (Radhakrishnan and Vijayakumaran, 1995). The larvae were reared individually and in mass culture systems. The temperature of the rearing system ranged from 26-29°C and salinity from 34-35 ppt. The mean total length of newly hatched larva was 1.48 mm and that of stage 6 was 4.87 mm. Attempts made at rearing phyllosoma larvae of *P. homarus* at the Calicut hatchery of CMFRI reached up to the sixth stage in 48-55 days with the mean length of 4.63 mm (unpublished). Later phyllosoma were

reared to stage 8 in 42 days on a mixed diet of *Artemia* and plankton (Radhakrishnan, 2012). Larvae of *P. ornatus*, *P. polyphagus* and *P. versicolor* were also reared through early stages.

Feeding is a critical factor in the rearing of phyllosoma larvae. Delayed feeding or decreased feeding may prolong intermoult period or cause death (Abrunhosa and Kittaka, 1997). The phyllosoma larvae are fed on *Artemia*, *Sagitta*, *Ctenophore medusa* etc. Initially the larvae feed on freshly hatched *Artemia* nauplii and in the later stages on mussel gonad, *Artemia* juveniles, *Sagitta* etc. *Artemia* is the most widely used feed item worldwide in the larviculture of fish and crustaceans (Van Stappen, 1996). They can be produced on a mass scale, are relatively small in size (450 µm), nutrient rich and the dormant cysts can be hatched on demand. Mussel gonad is a superior source of protein and lipid in comparison with *Artemia* nauplii. But it involves chopping, disinfectant steps which ultimately results in small product yield (Takeuchi and Murakami, 2007). The nutritional composition of mussel gonads also varies seasonally, hindering ability to provide guaranteed levels of nutrition to phyllosoma on a year round basis.

Farming

As mass scale production of pueruli/juveniles of spiny lobsters is yet to be accomplished, farming depends on the wild for seed. Studies conducted off Kovalam near Chennai show that pueruli of three species *P. homarus*, *P. polyphagus* and *P. ornatus* settle in rocky areas. There is no information on settlement density of pueruli anywhere along the Indian coast (Radhakrishnan, 2012). Tropical palinurid lobsters tolerate temperature fluctuations of 23-29°C. They show optimum growth in sea water of 30-38 ppt salinity and can adjust to low oxygen conditions. In India, on growing of juveniles in indoor tanks was developed by CMFRI and Tuticorin Fisheries College. Radhakrishnan and Vijayakumaran (1990) got a growth rate of 0.75 g by stocking juveniles of *P. homarus* in indoor tanks at a stocking density of 7 individuals/m². Sand filtered sea water with a salinity of 30-38 ppt, pH 7.8-8.4 can be used. Shelters should be provided. Studies in certain species of spiny lobsters have shown that shelters improve survival of juveniles. Chittleborough (1974) also found that *P. cygnus* consumed more

food and grew faster when shelters were provided.

The Central Marine Fisheries Research Institute conducted sea cage farming of spiny lobster *P. homarus*. In Vizhinjam, Southwest coast of India, growth of juveniles and sub adults of *P. homarus* were evaluated in land based FRP tanks and a large floating cage anchored at Vizhinjam Bay. The FRP tanks were stocked with 100 numbers of juveniles and reared for 120 days. The tanks had a water holding capacity of 10 l. In circular cages with HDPE frame and Poly Urethane foam (PUF) with a total volume of 110 m³ the lobsters were reared for 135 days. 1100 juveniles/sub adults were stocked and the cage was moored at a depth of 10 m, 75 m away from the shore in the Vizhinjam Bay (Rao et al., 2010). Specific growth rates of 0.45% and 0.50% of the body weight were obtained per day in FRP tanks and sea cages respectively. Better survival was obtained in the cage (75%) than in tanks (71%). *P. polyphagus* were reared in open sea floating net (18 mm mesh) cages of 6 m diameter at 8 m depth, 300 m away from Prabhas Patan, Veraval, Northwest India. In cage I lobsters 80-120 g were stocked and in cage II lobsters weighing <80 g. Cage II had a specific growth rate of 1.51% per day which was significantly higher than the specific growth rate of 0.80% per day in cage I (Mojjada et al., 2012), suggesting good potential for capture based aquaculture of the species in sea cages.

National Institute of Ocean Technology (NIOT), Chennai conducted sea cage farming of *P. homarus* using mild steel cages and reinforced plastic cages. The latter appeared to be better owing to higher durability and higher stability in unfavourable sea conditions (Vijayakumaran et al., 2009). Juvenile of < 90 g and sub adults 90-150 g from the regular fishery were stocked and fed once a day in the evenings. They were fed mainly on *Donax* spp. The gastropod *Xancus pyrum*, marine crab (*Charybdis* sp.), mantis shrimp (*Squilla* sp.) and squid (*Loligo* sp.), fish (*Clupeids* and *Leiognathus* sp.) and green mussel *Perna viridis* were also fed. Growth rate of 200 g in 365 days and 350 g in 490 to 520 days were obtained for *P. homarus*.

In Vietnam, sea cage culture of spiny lobsters (main species *P. ornatus*) was developed in 1992,

which significantly expanded in south-central Vietnam in 2000 (Hung and Tuan, 2009). Lobster sea cages grew rapidly here from 1999 and reached its peak (approximately 49,000 cages) in 2006. But the milky disease outbreak in late 2006 resulted in the decline of sea-cages to 47,000 and 41,000 in 2007 and 2008 respectively. Different types of cages are used in Vietnam depending on the characteristics of the culture area: floating cage supported by a frame and buoys (used at a depth of 10-20 m); wooden fixed cages made of salt resistant wood (used in sheltered bays); submerged cages of iron mesh framework (used for nursing juvenile lobsters and grow out farming). Most commonly used feed for the lobsters is *Saurida* spp; *Priacanthus* spp., *Leiognathus* spp; pomfret; snails, oysters, cockles, small swimming crabs and shrimps. Lobsters are fed 3-4 times / day. Wild caught juveniles are used for culture.

Research conducted at NIWA, New Zealand, has showed enormous potential for sea cage on – growing of *Jasus edwardsii* (James, 2007). A study on the effects of salinity on lobster growth and mortality found that both fluctuating (25ppt-35ppt) and low (25ppt) salinity treatments had significantly lower growth and higher mortality than 30 ppt and 35 ppt treatments. Following the commercial success, NIWA has continued research into both sea-cage design and the development of artificial lobster diets to improve the economic viability of lobster on-growing in sea-cages.

The main market for farmed lobsters is China, Hong Kong and Taiwan. China is the main export market especially for *P. ornatus*. Their larger size, beautiful colour and firm pearly flesh are perfect for serving raw as 'Sashimi'. The large producers and exporters of lobsters in the Indian and Pacific oceans are Australia, New Zealand and Indonesia. Potential for sea cage farming of spiny lobsters is being assessed in different countries. An industry equivalent to that in Vietnam is developing in Indonesia (Jones and Shank, 2009). In India, demonstration on cage farming of lobsters was successfully conducted in Tamil Nadu and Gujarat by CMFRI. This has opened up new vistas for alternate livelihood for the coastal fisher folk. The undersized lobsters that form an incidental catch in the fishery are used for farming. There is need for detailed survey to assess the pueruli/early post pueruli settlement areas.

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Seaweed farming

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Introduction

Seaweeds are leafless, stem-less and rootless plants that grow in the sea. The word seaweed gives the wrong impression that it is a useless plant. Seaweeds are wonder plants of the sea and highly useful plants. Seaweeds grow in the shallow waters. Root system and conducting tissues like land plants are absent in seaweeds. Most of them have hold-fast for attachment and some drift loose in the sea. Four groups of seaweeds are recognized according to their pigments that absorb light of particular wave lengths and give them their colours of green, blue, brown and red.

Seaweeds are marine macro-algae found growing throughout the world oceans and seas. Though there are about 9200 species of seaweeds, only 221 species are economically important. Over 68.33 lakh tons of brown, red and green seaweeds are exploited annually for the production of various commercially important phyco-colloids such as agar, algin and carrageenan. Thus, natural seaweed stocks have become inadequate to meet the industrial requirements and hence cultivation of these important resources has become necessary. Asia stands as the world leader in seaweed cultivation and more than 80% is contributed by China, Korea and Japan. India has not taken up seaweed cultivation interestingly in the past though it is bestowed with a coastline of more than 17,000 km, embracing 821 species of seaweeds. Only recently, seaweed cultivation is picking up in certain coastal districts of the Tamil Nadu state. Central Salt Marine Chemical Research Institute and Central Marine Fisheries Research Institute have developed culture techniques for some of the commercially important seaweed species in India. As a consequence to this, cultivation of *Eucheuma* and *Hypnea* has been taken up on a commercial scale. As a result of this effort, a lot of Self Help Groups, Village Youth Groups and NGOs have come forward to promote seaweed cultivation as an alternate livelihood option for the coastal poor. Considering the great demand for these resources in the international market and

availability of adequate manpower and interest in the country, seaweed cultivation has a very good prospect and it can be developed as a successful cottage or co-operative sector industry.

In India, mariculture is a sunrise enterprise. Technologies that have attracted the imagination of coastal stakeholders include mussel farming, seaweed farming and sea cage culture. Mussel (*Perna viridis*) farming technology has diffused along the Malabar coast (southwest India), and seaweed (*Kappaphycus alvarezii*) farming prevails along the Coromandel coast (southeast India), after it found a niche in the Gulf of Mannar. Having proven their potential as empowerment platforms for coastal women, the theatres where these technologies were adopted raised a number of issues in the realm of a gendered political ecology. The aim of this paper is not only to diagnose these issues but juxtapose them with some of the epistemological concerns being brought by “gender lens” scholarship, especially in the neo-liberal context of global fisheries. A paradox brought out by the present study is the ambivalence of the State in manifesting itself as a positive “bargaining” force in the intra-household domestic space (by providing State-sponsored platforms through the Self Help Groups) while leaving the “common access resource” space, from which these platforms gain sustenance, less amenable to its democratic ideals.

Uses of seaweeds

New renewable source of food, energy, chemicals and medicines

Create opportunity for employment

Provide valuable source of raw material for industries like health food, medicines, pharmaceuticals, textiles, fertilizers, animal feed etc.

Seaweeds used for production of Agar, Alginates & Carrageenan.

Why seaweed farming

Remedy for non-availability of required quan-

tity of seaweeds for various uses.

Provide occupation for the coastal people.

Provide continues supply of raw material for seaweed based industry.

Provide seaweeds of uniform quality for use in industry

Conserve natural populations of concerned seaweeds

Ecofriendly activity

Major tool to treat coastal pollution in the sea and reduce CO₂ in global warming

Gracilaria farming

Gracilaria spp. can be cultivated using vegetative fragments. Vegetative fragment culture of Gracilaria easy practice and it can be carried out throughout the year. Vegetative fragments of the plants are divided into 5 cm and these are introduced between the twists of the rope at 10 cm intervals. Fixed off bottom long line or floating raft methods can be selected. In the fixed off bottom long line method seaweed inserted ropes were tied to the posts planted in the sandy and muddy bottom of the intertidal regions..The position of the ropes is adjusted to remain at a constant depth in the tidal zone. In the raft method vegetative fragments inserted ropes were tied to the floating raft. First harvest can be made in three months and subsequent harvest in one and months. After harvest it may be dried in beaches itself for a week and kept in bales ready for shipping.

Kappaphycus farming

The farming of the seaweed Kappaphycus can be a low-cost venture and a profitable one, with the right site. The technology can use family labor in either fixed off-bottom or single raft long-line culture. The more line modules, the more investment and care are needed. After tying seaweed plantlets or "seedlings" to the ropes, and the ropes staked to the sea bed by bamboo or tied to floating rafts staked to the sea bed, seaweed farming needs no more inputs. There is periodic visitation, two to three times a week, to remove undesirable algae, barnacles, and attached sediments; to re-tie loose or fallen seaweed; to tighten lines; and to check for signs of "ice-ice" disease. Seaweed culture can last 45-60 days. In one Hectare 900 rafts can earn a net annual income of Rs. 4,60,000/- as-

suming per raft yields 280 kg of fresh seaweed per raft after grow out period of 45 days.. The ratio of fresh and dry seaweed is 1:10 and the price is Rs. 16/- per kg of dry seaweed (CMFRI Special Publication No. 104).

Technology profile

Get and select good quality seedlings; these are brittle, shiny and young branches with sharp pointed tips, no traces of grazing or whitened thallus (sign of beginning "ice-ice" disease), and 100-150 g.

For fixed off-bottom culture: while on land, tie seaweed seedlings 15-20 cm apart to the cultivation rope 10-20 m long with soft plastic string (commonly called "tie-tie"). Carry the ropes to the site at the lowest tide and tie both ends to stakes already placed 1-meter apart on the seabed. For single raft long-line ~ Tie seedlings as above but anchor ropes to a bamboo raft. A raft unit consists of four bamboos in a square arrangement as support with two ends tied in turn to anchor lines which are staked to the seabed. A longer raft long-line (50-70 m long) can be made; floats are regularly spaced in this instance to add buoyancy to the raft. In deeper waters (5-10 m), the hanging long-line may be best; less bamboo support is used but a good concrete block anchor is necessary.

Visit the farm two to three times a week. Remove undesirable algae, barnacles, or attached sediments. Re-tie loose or fallen seaweed. Check and tighten loose rope or stake. Check for signs of diseases; totally harvest crops immediately if present. Use new set of seedlings, change farming site / method, and use lower stocking density.

Harvest in 45-60 days. Seaweed can be sold wet or dry to processors. Dried seaweed brings more income if it is clean and with moisture content of 35-39%. It is best to keep harvested seaweeds off the ground (remember that the carriage is bound for products for human consumption). Use a layer of mat, fish net, or coconut leaves and constantly turn seaweeds to accelerate drying; or dry seaweeds in a platform or hangings lines. Sun-dry for 2-3 days.

Tie the seaweed in bales, then store in a clean, cool, dry and well-ventilated place while awaiting buyers.

Why Kappaphycus farming

High return on investment

Demand for seaweeds is high in the local and international markets

Culture period could be as short as 45 days under optimal conditions

Environment-friendly

Could be a source of supplemental income for small fisherfolk associations and people's cooperatives

The farming of the seaweed *Kappaphycus* can be a low-cost venture and a profitable one

Conclusion

Seaweed farming based primarily on the culture of *Kappaphycus* species has grown significantly

in the Philippines and Indonesia over the last two decades, with growth also taking place at a smaller scale in India and a few other developing countries. Unlike other forms of aquaculture, seaweed farming foregoes the use of feed and fertilizers and has minimum technological and capital requirements. In addition, grow out cycles are short, normally lasting less than two months. Given these unique characteristics, seaweed farming has generated substantial socio-economic benefits to marginalized coastal communities in developing countries, most of which have reduced access to alternative economic activities. In some communities, seaweed farming has emerged as the most relevant livelihood strategy. Given the rising global demand for seaweed-derived products, seaweed farming has the potential to generate further socio-economic benefits to coastal communities in tropical regions.



Vegetative fragment of Kappaphycus



Kappaphycus farming by floating raft culture method



Fixed off bottom long line method of Kappaphycus farming in the lagoon



A long view of Kappaphycus farming by adopting fixed off bottom method



Harvested Kappaphycus



Harvested Kappaphycus along with raft

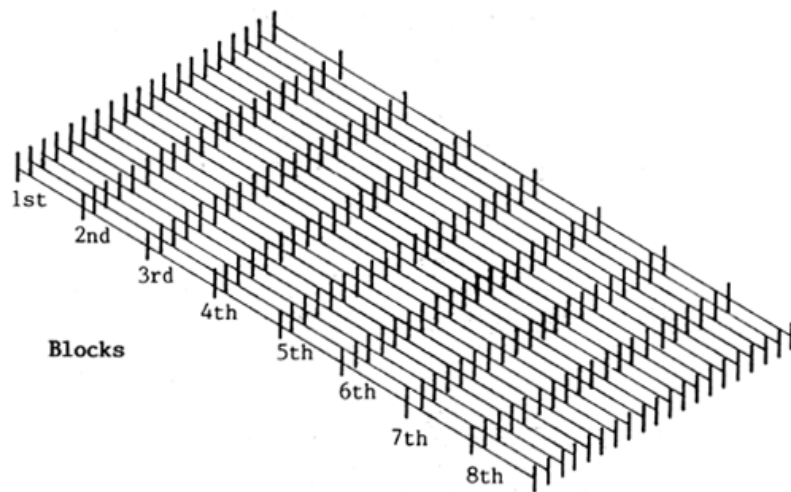


Diagram showing fixed off bottom seaweed farming method

Exotic fish culture: Indian experience

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India is having about 2.5 million ha area of potential freshwater resources in the form of ponds, reservoirs and tanks, but utilise only about 42% area at present for fish culture. With production of over 3.3 million tonnes from the inland fisheries sector, the country has occupied the second position in the world. Such growth has been possible due to impressive developments in aquaculture, mainly through carp culture in freshwater ponds and tanks as also the integrated fish farming practices. During the past two decades, the inland aquaculture fish production has increased to 2.9 million tonnes, with carps alone contributing over 85 per cent. However, the average national productivity from these cultured areas is about 2 tonnes/ha/yr, despite the availability of technological know-how for achieving higher production levels. Thus, there is a great scope for increasing the production through adoption of scientific grow-out technology and expansion of culture area. Right kind of seed, feed and the fertilisers form the three critical inputs, while their proper management decides the success of the grow-out production. Further, management of soil and water quality and fish health aspects also contribute to a great extent. Catla, rohu and mrigal, known as Indian major carps, are the important carp species cultured traditionally in ponds and tanks due to their higher growth and consumer preference. The exotic silver carp, grass carp and common carp having higher growth potential are also added along with these three Indian major carps for increasing the production under composite carp culture. Though initial years tilapia was introduced for stocked in reservoirs for increasing productivity, later chinese carps were introduced. Latest, illegal introductions are African catfish, *Clarias gariepinus*, Malaysian catfish, *Pangasianodon hypophthalmus* and red-bellied pacu, *Piaractus brachipomus*.

Exotic Fish Introductions in India

Exotics are defined as “species occurring outside of its natural range”. Aquaculture and ornamental fishery are the main motives of the

introduction of a species in to a country. Exotic introduction of exotic fish to India during early 20th century were largely due to the effort of British rulers for development of game fishing. During the period 1870-1947 under the British rule, 9 species of exotic fishes were introduced. They were temperate food carps, *Tincatinca*, *Carassius carassius*, *Cyprinus carpio* (European strain), and the tropical osphronemid, *Osphronemus goramy*; the salmonid game fishes, the brown trout (*Salmo trutta*) and the rainbow trout (*Salmo gairdneri*); and larvicidal *Gambusia affinis* and *Lebistes reticulatus*. The Post-Independence, India witnessed further introductions of exotic species. They were the cyprinids, *Cyprinus carpio* (Chinese strain). *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Puntius javanicus*, and the cichlid, *Tilapia mossambica* all of food species; and the salmonids, *Salvelinus fontinalis*, *Onchorhynchus nerka* and *Salmo salar*. *Aristichthys nobilis*, *Tilapia nilotica* and red tilapia. Recently in India, genetically modified fish tilapia (GIFT) and all male strains of tilapia were legally introduced by Rajiv Gandhi Centre for Aquaculture (RGCA), MPEDA, and Government of India for selective breeding and restricted propagation of GIFT among selected farmers. In recent years, many fishes introduced illegally, which includes, African catfish, *Clarias gariepinus*, Malaysian catfish, *Pangasianodon hypophthalmichthys* and red-bellied pacu, *Piaractus brachipomus*. In 2010 Malaysian catfish, *Pangasianodon hypophthalmichthys* was given legal status for introduction in the country. In the brackish-water sector, after a debacle of WSSV in *Penaeus monodon* culture, white Leg Shrimp *Litopenaeus vannamei* was introduced which gave remarkable profit to farmers.

Benefits of Exotic fishes

Tilapia culture-based experiments were carried out in India on several places. The fish, in intense culture, with common carp and catla resulted in a production of about 5,000 kg/ha/yr; its share was about 300-330 kg/ha/y in composite fish culture (Alikunhi et al., 1971); 1200 kg/ha/yr

in monoculture. The fish has extensively spread in sewage-fed bheries in West Bengal.

Carp:

A mixed carp-farming practice made of three exotic and three indigenous species of major carps was developed (Alikunhi et al., 1971). In this farming practice, the species spectrum is limited to two trophic levels; primary consumers and secondary consumers. The silver carp, grass carp, rohu and mrigal are primary consumers, catla a secondary consumer, while common carp is both primary and secondary consumer. In intense composite culture a production range of 6000-9000 kg/ha/y was achieved (Chaudhury et al., 1975; Chakraborty et al., 1979). Under the All India Coordinated Research Project on composite carp farming at intensive and semi-intensive levels the production was in the range of 3,500-10,000 kg/ha/yr (Sinha, 1976). The higher production level achieved in six species composite carp farming is largely due to major share of exotic fishes among stocked material and higher growth rate and thus appears more physiological than ecological. The bioenergetics of exotic carps are governed by a higher metabolic activity i.e., temperature induced higher rate of ingestion and a corresponding higher growth of temperate.

Catfishes:

India witnessed introduction of three catfishes, Channel catfish, *Ictalurus punctatus*, African catfish, *Clarias gariepinus* and Malaysian catfish, *Pangasianodon hypophthalmus*. Channel catfish was introduced for aquaculture by M/s Hindusthan Lever Ltd., but failed the culture. African catfish was introduced in early 1990s and Malaysian catfish introduced in 1990s. Both of the fishes were illegally introduced, but later in 2010 *Pangasianodon hypophthalmus* was legalized in India. Because of its remarkable growth rate (almost one kg in 90 days), there has been much enthusiasm among fish breeders and farmers particularly in West Bengal and Andhra Pradesh for its culture and propagation. It is estimated that over 200,000 tonnes of *P. hypophthalmus* catfish are produced in the country per annum. The total area of fish culture of *P. hypophthalmus* was estimated to be over 20,000 ha covering roughly 15% of the total culture area, which has increased over the years. Due to closure of shrimp ponds on account of disease, farmers suffered heavy losses and many adopted *P. hy-*

pophthalmus farming as an alternative crop in the same areas that were disease affected. The state of West Bengal was found to be the hub of seed production of *P. hypophthalmus* in the country. About 300 to 500 million *P. hypophthalmus* seed is produced every year with the bulk of it being sent to Andhra Pradesh and the rest to Orissa, Tamil Nadu, Maharashtra, Kerala, Karnataka, Bihar, Rajasthan and Uttar Pradesh. The seed production of *P. hypophthalmus* is not only used for aquaculture but is also sold for the aquarium trade. Different varieties of fingerlings (striped and albino) are produced for aquarium trade. The culture production of *P. hypophthalmus* ranges from 7 tonnes per hectare per year to 20 tonnes per hectare per year and the average production are found to be higher than carp production in the same areas. Today in all over the country, Malaysian catfish is being cultured mostly as monoculture. The production levels are 10,000 to 20,000 kg/ha/yr under good management conditions. However, the culture of *Clarias gariepinus* is minimised due to non-preference of the consumers in the market. Recent survey shows that more than 200000 ha area is under Malaysian catfish culture in Andhra Pradesh alone.

Litopenaeus vannamei

White leg shrimp *Litopenaeus vannamei*, native to the eastern Pacific Ocean, was introduced in India following the debacle of WSSV in *Penaeus monodon* after 1995. This species spread in the aquaculture, as it was not showing any disease problem. At present this shrimp has helped increase India's total seafood exports by five per cent in quantity and 12 per cent in profits. The country exported a record 91,000 tonnes of vannamei shrimps (40,787 tonnes in the period between 2011 and 2012). Frozen shrimps, including other varieties, constitute 50 per cent of the total value of total seafood exports, and India exported 189,000 tonnes of the produce, earning Rs. 80,000 million. According to the Marine Products Export Development Authority (MPEDA), seafood export figures for the last financial year are yet to be revealed. However, in the previous fiscal year, India exported 862,000 tonnes of seafood, valued at about US\$3.5 billion. One of India's leading seafood exporters said vannamei is still relatively new to the country but despite its novelty, it has made a positive impact on the seafood business for farmers and exporters alike. The vannamei shrimp made a

resounding entrance into the Indian seafood market with high productivity and export figures have been impressive since 2010-2011 when the country exported 12,407 tonnes. The total area under vannamei farming is 22,715 hectares. However, seafood exporters, many of whom are involved in aquaculture, estimate that there may at least be 50,000 hectares under farming (MPEDA). The production of *L. vannamei* was 1,23,551 tonnes in 2012-2013 compared with 80,717 tonnes during 2011-12, a growth of 53.07 per cent.

Other exotic fishes

Though many trout species introduced in India, culture of these species is very limited to Jammu Kashmir and Himachal Pradesh. In Himachal Pradesh trout hatcheries were developed and running water aquaculture is well established. In many places trout farming is still at initial stages of development. *Osphronemus goramy* is another species of interest in culture but not many of the farmers taken this species for culture due to its slow growth rate. During 1970s *Puntius (Barbodes) javanicus* was introduced in India and got a place in rice cum fish culture. Later breeding and seed production was perfected for this species and culture practices also perfected. However, culture of this species is not spread among farmers due to non-availability of seeds in farmers' site.

Utilization of Exotic Fishes

The country has an estimated 3 million ha of water area at FRL under reservoirs in which the blue green algae, microcystis, are the dominant phytoplankton. We have no freshwater fish of economic importance that are physiologically well-equipped to exploit this ecological niche. But African cichlids are apparently well equipped to utilize this ecological niche, which perhaps is the one major factor contributing to a higher yield rate in reservoirs in Ceylon (Fernando, 1969). Vast network of canals has been playing a crucial role in irrigated agriculture in India. These canals suffer from impeded flow by aquatic weeds that choke the canal bed. The Indian species of fish are not found adequate to control the spread of the weeds. The Chinese grass carp, an effective macrophagous herbivore, is an excellent bioengine for this task. *Tilapia* spp. belonging to macrophagous herbivore category is also endowed with similar traits. The modernised agriculture

employs extensively plant nutrients, part of which is drained into open waterways. Similarly sewage, the magnitude of which is alarmingly increasing in proportion to rise in population growth and rate of urbanisation is another major source of nutrient loading especially phosphorous and nitrogen that had resulted in artificial eutrophication in many of our hill lakes, floodplains lakes, river ways and lagoons. The microphagous and macrophagous herbivores like African cichlids and Chinese carps are known to mitigate eutrophication. They are also excellent material for stocking oxidation ponds. The exotic cichlids are also known to utilise distillery and brewery wastes and thus help in pollution abatement.

Physiological role of exotic fishes in fish husbandry: As pointed out elsewhere in this paper, eurythermal temperate species of exotic carps have physiological potential for high growth rate when cultured in elevated water temperature up to a point in tropics. This is the principal factor for higher production rate achieved under composite fish culture and not ecological. In fact field observations point to inter-specific competition between exotic and indigenous carps, an ecological offshoot when ecological homologues are integrated. The Indian combination of catla, rohu, mrigal and kalbasu are ecologically compatible tropical species. The Chinese combination of silver carp, grass carp, big head, mud carp, black carp and bream are similarly ecologically compatible temperate species.

Conclusion

Though many introductions of fishes have become beneficial in terms of productivity and production, we have to take precaution before introducing the fishes into a country. Many fishes become invasive in the introduced country like tilapia in India. Tilapia, being an omnivore, eats everything and also competes with native fishes, thus reduce the population of native fishes. Those fishes, which are having more physiological capacity to digest the food particles take a dominant stage and will pose a threat to the biodiversity of that area. There are chances of introducing many exotic diseases along with the fishes. Hence, before introducing the exotic fishes, proper risk assessment need to be done with respect to the native fishes of the country and compatibility with native fishes.

Economic considerations in mariculture

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Introduction

Fisheries sector serves as important source of animal protein a potential employment and income generation besides earning substantial foreign exchange through the seafood exports in most of the developing countries. In India, for e.g fisheries contribute consistently about 5% of India's agricultural GDP and about one per cent of the total GDP during the last five years. The sector also supports the livelihood of about 14 million populations. When the production (harvest) from marine fisheries reaches a stagnation phase, with limited scope for further expansion, the alternative is to look for augmenting the fishery resources of the sea. Looking into the Sea is an important alternative available in front of us. Among the many alternatives available like sea ranching, artificial reefs, mariculture is one of the potential alternatives, which can be practiced by the fishermen more effectively. Mariculture systems include in-shore and off-shore and maintain a constant high saline water conditions. In-shore mariculture systems include clams, oysters and other molluscs, which are wild-caught or hatchery-reared seed grown on the sea floor or on suspended nets, ropes, or other structures (Naylor 2001). Off-shore mariculture refers to large intensive fisheries in off-shore fish pens.

Mariculture: Example from Indian experience

Mariculture has the potential to augment production and incomes through coastal as well as open sea farming. The global aquaculture production increased by a about 25 times in the last 30 years against only seven times increase in capture

fisheries production during the corresponding period (Gopakumar et al., 2007). India has vast areas of suitable coastal waters, lagoons and bays which can be utilized for mariculture. Seed production and culture of marine finfishes has been expanding in the recent past in many parts of the world, but in India, it is only an emerging sector. The potential cultivable candidate finfishes are groupers, cobia, rabbitfish, seabass, pompano, snappers and sea bream. Lack of availability of hatchery-produced seed on a commercial scale is the major bottleneck for large-scale marine finfish farming. The availability of seed from wild is often unpredictable, and hence, the development and standardization of seed production techniques for a few commercially important species is receiving research priority.

Economic analysis of mariculture

The success of the adoption of any innovation or new technology lies in its economic performance. The rate of return per rupee invested is the economic indicator that guides the investor to choose a particular enterprise or practice. Besides, the analysis of the economic performance serves as an indicator for the investor to allocate his resources in the enterprises. This becomes very much essential, since the resources are scarce and the investor is interested to invest his scarce capital resource in that enterprise that gives the maximum return for his investment.

The economic performance of any mariculture activities can be assessed by working out the following cost and return indicators and financial feasibility of any enterprise. (Narayanakumar, 2009, Sathiadhas & Narayanakumar, 2010)

Table 1 Indicators of economic performance of a mariculture enterprise

Sl.No.	Economic Indicators
1	Initial investment a)Fixed installations b)Land (if any)

	c) Major accessories
	d) Minor Accessories
	e) Others
2	Fixed cost (For crop duration of six months)
	a) Depreciation
	b) Insurance (2% on investment)
	c) Interest on Fixed capital (12%)
	d) Administrative expenses
3	Total Annual Fixed cost (A)
4	Operating costs
	a) Cost of seedlings
	b) Cost of feeding and other labour charges
	c) Interest on working capital (6%)
5	Total Operating or Variable cost (B)
6	Total cost of production [Row(3) + Row(5)]
7	Yield of the fish variety (in kg)
8	Gross revenue [(7) * Price per kg]
9	Net income [(8)-(7)]
10	Net operating income [(8)-(5)]
11	Cost of production (Rs./kg) [(6)/(7)]
12	Price realized (Rs./kg) (8)/(7)
13	Capital Productivity (Operating ratio) (5)/(8)

As seen from the table, the different economic indicators of the economic performance of any mariculture enterprise are worked to assess its performance. This will serve as the guidelines to the institutional agencies that are extending the financial support to the enterprise. We can see some of the case studies in mariculture conducted by CMFRI to explain the economic considerations in Mariculture

Case studies

1. Cage farming at Balasore, Orissa

Farming in open sea cage farms is an alternative practice with great potential to increase production of high value edible finfish and shellfish. In recent years, open sea cage farming is expand-

ing on a global basis. In India, the sea bass was cultured by CMFRI in cage diameter: 6 m; depth: 6 m off Balasore near Orissa in a demonstration project. The cage was launched near Chaumukh beach in Balasore during January, 2009 and was stocked with 4,357 numbers of locally collected Asian seabass juveniles. After about six months, around 3,200 kg seabass was harvested indicating the potential. The cost of production per kg of sea bass worked out to Rs.94.24/- against the value realization of Rs.189.89 per kg. The capital productivity measured through operating ratio worked out to 0.80. These economic parameters indicate that this open sea cage farming of sea bass is economically viable (Table 2). (Rao et.al., CMFRI, 2009)

Table 2 Economic analysis of the experimental cage culture demonstration at Balasore

Sl.No.	Details of cost and returns	Amount (in Rs.)
1	Initial investment for a 6m diameter cage	3,00,000
2	Fixed cost (For crop duration of six months)	30,000

	a) Depreciation	3,000
	b) Insurance (2% on investment)	18,000
	c) Interest on Fixed capital (12%)	3,000
	d) Administrative expenses	
3	Total Fixed cost (A)	54,000
4	Operating costs	50,000
	a) Cost of seedlings	1,75,000
	b) Cost of feeding and other labour charges	6,750
	c) Interest on working capital (6%)	
5	Total Operating cost (B)	2,31,750
6	Total cost of production (Six months)	2,85,750
7	Yield of sea bass (in kg)	3,032
8	Gross revenue from 3032 kg	5,75,760
9	Net income (8)-(5)	2,90,010
10	Net operating income (Income over operating cost)	3,44,010
11	Cost of production (Rs./kg) (6)/(7)	94.24
12	Price realized (Rs./kg) (8)/(7)	189.89
13	Capital Productivity (Operating ratio) (5)/(8)	0.5

2. Cage farming at Visakhapatnam

Table 3 Initial investment of the cage culture farm of 1061 m³

Sl.No.	Items	Investment (in Rs.)	% to total	Economic life (in years)
1	HDPE Cage frame	4,00,000	27.12	10
2	HDPE nets	3,00,000	20.34	10
3	Galvanized Iron Chains	80,000	5.42	10
4	Mooring equipments	60,000	4.07	10
5	Stone Anchors	1,50,000	10.17	50
6	Floats	1,50,000	10.17	10
7	Shock absorbers	25,000	1.69	10
8	Ballast	35,000	2.37	10
9	Ropes-HDPE	35,000	2.37	10
10	One time launching charges	2,40,000	16.27	
	Total Initial Investment	14,75,000	100	

Table 4 Details of Annual Fixed cost

Sl. No.	Details	Amount (in Rs.)
1	Depreciation	1,16,000
2	Insurance premium (5% of investment)	73,750
3	Interest on fixed capital	1,77,000
4	Administrative expenses (2%)	29,500
	Total fixed cost	3,96,250

Table 5 Details of Annual Variable cost of cage culture (for a crop duration of seven Months)

Sl. No.	Details	Cost	% to total
1	Feeding	2,24,000	14.02
2	Seedling	1,50,000	9.39
3	Feed cost	9,00,000	56.32
4	Net cleaning	75,000	4.69
5	Underwater inspection	50,000	3.13
6	Net mending and Maintenance	25,000	1.56
7	Post crop overhauling	20,000	1.25
8	Security	1,00,000	6.26
9	Interest on working capital @6% for one crop duration	54,040	3.38
	Total	15,98,040	100.00

Table 6 Economic indicators of the cage culture of Lates calcarifer, Visakhapatnam

Sl.No.	Details	Amount (in Rs.)
1	Annual fixed cost	3,96,250
2	Annual Variable cost	15,98,040
3	Annual total cost	19,94,290
4	Gross revenue (after harvesting from 5th to 7th month)	37,50,000
5	Net operating income	21,51,960
6	Net income (profit)	17,55,710
7	Capital Productivity (Operating Ratio)	0.43
8	Annual Rate of return to capital (%)	119%

Thus it is seen from the above results that the economic analysis of the experimental cage culture farm has worked out successfully with higher net operating income and net income in a crop period of seven to nine months. It is to be noted that once the practice is further expanded to many areas and farms, the cost will decline due to the economies of scale of operation. Thus it could be concluded that the open sea cage farming is a viable alternative and economically & financially feasible mariculture operation for the stakeholders to make use of in the developing countries.

Mariculture: A potential source of employment

The mariculture has proven to an economically viable alternative to augment the biomass production from the seas in situations wherever the fishery resources are harvested beyond the sustainable limit. Looking into the seas is the key word for increasing the fish production from the

sea as well as improving the livelihood of the million people who depend on the sector.

The mariculture activities provide adequate employment opportunities for the fishers to sustain their livelihood. An estimate by Syda Rao and Gopakumar (2010) indicated that the open sea cage farming of a species provide 1,040 man days of work; open sea lobster farming, 496 man days; mussel culture -52,000 man days; oyster farming-30,000 man days and seaweed culture 3.06 lakh man days. From these estimates, the scope of commercial mariculture can be understood.

Conclusion

Mariculture and research in mariculture is in different stages of development in different countries. The increasing awareness of the consumers on the shell fishes like clams, oysters & mussels and increasing interest for the cultured high valued fin fish can be capitalized by adopting and

investing on taking up mariculture practices. This will help the commercial mariculture to develop to greater heights besides contributing to the food security of the country and providing consistent remunerative livelihood to the fishing community.

It is also equally important to see that the fishermen are given rights to farm in the open sea and its legal implications. There should be a strong policy back up for the establishment of such enterprises to enable the fishers to carry on their mariculture activities. A comprehensive policy framework to patronize the mariculture enterprises is the most essential step in promoting mariculture in any country. This supported by a systematic research programme on mariculture will help the country interested in developing mariculture to reach greater heights in the field.

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Role of self help groups in mariculture

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Introduction

The 'Self Help Group' (SHG) concept exists prior to any intervention. The SHG consists of members linked by a common bond like caste, sub-caste, community, place of origin, activity etc. in these 'natural groups' or 'affinity groups'. The 'Self Help Groups' provide the benefits of economies in certain areas of production process by undertaking common action programmes like cost effective credit delivery system, generating a forum for collective learning with rural people, promoting democratic culture, fostering an entrepreneurial culture, providing a firm base for dialogue and co-operation in programmes with other institutions, possessing credibility and power to ensure participation and helping to assess the individual member's management capacity (Fernandez, 1995). The open access regime existing in the harvesting of marine fishery resources in our country warrants stronger emphasis on invoking technological innovations as well as management paradigms that reconcile livelihood issues with concerns on resource conservation. Being the premier Marine Fisheries Research Institute in India with more than 6 decades of service to the nation, the Central Marine Fisheries Research Institute (CMFRI) suggests ways and means to sustain the potential source of food in capture and culture fisheries and their optimum utilisation. Innovations do not happen in a socio-political vacuum. It is the extent of partnership between the research and the client system that decides the fate of any technology in terms of its adoption or rejection. Rational utilization of common property resources for sustainable development without endangering the environment is possible through community participation.

Meaning of a micro enterprise

A micro enterprise is an activity which requires less capital, less manpower, local raw materials and local market. It is an individual enterprise whether known or unknown. (Vedachalam, 1998) In fisheries sector, for the upliftment of fisherfolk

below the poverty line, some successful micro enterprises developed based on the location specific resource availability and experience and some alternate avocations and subsidiary entrepreneurial ventures successfully being undertaken by Microfinance Institutions in coastal sectors and allied areas as follows: Value added fish producing units, Dry fish unit, Fish Processing unit, Ready to eat fish products, ready to cook fish products, Ornamental fish culture, Mussel culture, Edible oyster culture, Clam collection etc. are very important. In agricultural sector, Vegetable cultivation, Ornamental gardening, Floriculture, Kitchen Garden, Orchards, Fruit products, Fruit processing, Sericulture, Mushroom cultivation, Medicinal Plants, Vermi compost, Snacks units, Catering Units, Bakery Units, Cereal Pulverizing units are some micro enterprises undertaken by Self Help Groups.

Based on the resource availability and circumstances the micro enterprises those the SHGs' can generally bring to practical utility in allied sectors are Wood work units, Stone work units, Soap units, Garment units, Computer centre, Poultry centre, Cattle rearing, Piggery unit, Bee Units, Stitching units, Hand Weaving Units, Candles, Chalks, Umbrella units, Foam Bed Units, Bamboo based handicrafts, Paper cover, Scrape selling, Vegetable seeds, Marriage bureau, Medicine collection, Patients service, Real estate, Medicine processing, Direct marketing, Coir Brush, Plastic weaving, Second sails, Meat masala, Rasam powder, Curry powder, Pickle powder, Sambar powder, Consumer service centres, Home delivery package, Repacking business, Cleaning powder, Phenol lotion, Liquid soap, Washing soap, Toilet soap, Kids' garments, Toffee & Sweets, Photostat, Washing powder of best quality and medium type, Emery powder, Domestic animals, Nursery plants, Note book, Book binding, Rubber slipper production, Pillow cushion, Incense stick production, Cloth whiteners, Eucalyptus oil, Dolls, Hand shampoo, Soap shampoo, detergent shampoo, Jackfruit jam, Chips, Hotel, Catering service,

Grape wine, Pineapple wine, Soft drinks, Chicken farming, Dried mango wafer, Dried chilli, Gooseberry wine, Ginger wine, Pappads, Tomato sauce, Day care centre, Coconut water vinegar, Syrups, Artificial vinegar, Mixed fruit jam, Milk chocolate, Tomato squash, Gum production, Cleaning lotion, Soft drink shop, Reading room, Private tuition, Counseling-guidance, Rent sales, Paying Guest service, Repairing centre and handicrafts are some of the employment opportunities that the SHGs' can venture throughout Kerala depending on the suitability of situations and availability of resources. The suitability of the enterprise varies from situation to situation. The essential features for the success of a viable micro enterprise are :

The availability of sufficient quantity of raw materials locally.

The identified enterprise is known or easy to learn and practice.

The cost of production must be low.

The products must be of very good quality.

The availability of market for the products.

The present study focuses on the relevance of mariculture successfully attempted by SHGs. Mariculture offers good scope for development in our open waters for enhancing food and livelihood security of the stakeholders in our coastal agro climatic zones. The micro enterprises suitable in fisheries sector for SHGs in this sector are Mussel culture, Edible oyster culture, Pearl culture, Seaweed culture, Cage culture etc. Mussel farming has already been proved as one of the profitable enterprises in the coastal belts as a subsidiary income-deriving source of coastal fisherfolk. The experimental trials conducted by CMFRI have proved the techno-economic feasibility of mussel farming. (Asokan et al, 2001, Vipinkumar et al, 2001, Vipinkumar and Asokan, 2008). Here an attempt has been made on exploration of three case studies in Kasargod and Kollam districts of Kerala and Karwar of Karnataka on dynamics of Self Help Groups of fisherfolk engaged in Mussel Farming. Experiences and observations indicate that, for a group to be developed as a Self Help Group, normally a period of 36 months (3 years) will be required. Within this gestation period when the group passes through three distinct phases, up to 4 months as the Formation Phase, up to 15 months as Stabilisation Phase, and up to

36 months as the Self Helping Phase, the group gets led to the stage of a flourishing Self Help Group as per the indications given by social research results on Self Help Groups. The three distinct phases and the critical features are described as follows:

Group Initiation / Formation Phase (0 to 4 Months)

The major steps in this phase should include the initial visit to the location, rapport building, awareness creation, identification of women fisherfolk, conduct of meetings, documentation of deliberations, action plans for arranging raw materials for the fishery based and diversified micro enterprises and the selection of 'leader of fisherwomen'

Building up / Stabilization Phase (4 to 15 Months)

This phase must involve regular fortnightly meetings, maintenance of documents, scheduled implementation of action plan, procurement of inputs based on procurement plan as per production plan prepared based on market demand, market synchronized production planning, intensive training to carry out activities of production, credit and marketing aspects and changing the leaders of SHG after one year so that periodic rotation gives the other potential leaders a chance to lead.

Self Helping Phase (15 to 36 Months)

The main steps to be included in this phase are the development of a fortnightly action programme, meetings for sharing experiences, refinement, and improvement and problem solving for the activities under the responsibilities of the leaders, The extension personnel's role will be limited to that of a facilitator, gradually reducing their presence at meetings. Active leaders will give way to new leaders after a two year term; inter-SHG contacts and healthy competition will be encouraged, favorable group atmosphere, empathy and interpersonal trust for significant achievements of SHG will be encouraged.

The fisheries Self Help Groups have to focus attention on joint efforts co-operatively for finding out suitable micro enterprises, which can assure a constant income for the fisherfolk, based on locally available resources for poverty eradication. The Group Dynamics of these SHGs refer to

the interaction of forces between the members. It is the internal nature of the groups as to how they are formed, what their structures and processes are, how they function and affect the individual members and the organization. (Lewin et al. 1960). In an intensive study of Group Dynamics, Pfeiffer and Jones (1972) identified the Group Dynamics factors as to how the group is organised, the manner in which the group is led, the amount of training in membership and leadership skills, the tasks given to the groups, its prior history of success or failure etc. In a detailed study of Group Dynamics, Hersey and Blanchard (1995) gave emphasis on helping and hindering roles individuals play in groups such as establishing, aggressive, persuading, manipulative, committing, dependent, attending and avoidance. A couple of case studies on dynamics of Self Help Groups engaged in mussel culture are explored here.

1. Case study on Mussel Farming Self Help Groups of Women in Kasargod district

The extreme north district of Kerala named as Kasargod, is particularly notable for mussel farming as it has been successfully accomplished by the women's Self Help Groups (SHGs) for the past few years. These groups were given financial assistance in the scheme namely; SGSY (Swarnajayanthi Gramaswa Rosgar Yojana) by the state government which takes care of economic empowerment of weaker sections (Vipinkumar et al 2001). Subsidies, bank loans etc are the part and parcel of it and it essentially focuses attention on poverty alleviation through organised Self Help Groups. This programme looks into training, credit, marketing, technical knowledge and basic facilities necessary for the upliftment of the poor to bring them above the poverty line within three years in such a way that they should have a monthly earnings of at least Rs 2000/-. It would be pertinent to have a look into the consequences of adoption and cost dynamics of mussel farming by the women's Self Help Groups in Kasargod district.

This district possesses an area of 1992 km² with a population of 10, 71,508. The district with a population density of 538/km² has an average growth rate of 22.78 and 82.51 % literacy rate. Majority of the villagers earns their livelihood by agriculture, fishing, coir retting, coconut husk, toddy tapping etc. There is tremendous poten-

tial for aquaculture diversification in Kasargod coastal belts. Water bodies in these coastal belts have ample scope for the judicious utilisation of finfish culture, prawn and crab farming in Kasargod. (Asokan et al 2001).

This study was undertaken in two major panchayaths namely Cheruvathur and Padanna in Kasargod district. The study area, Cheruvathur panchayath has an area of 18.37 km² with a population of 24, 504 out of which 18, 631 people are literate. Agriculture is the main occupation of the majority and about 150 families are engaged in fishing as the main occupation and about 300 families as subsidiary occupation.

Similarly, Padanna panchayath has an area of 13.08 km² with a population of 17, 961 out of which 12, 746 people are literate. About 200 families are engaged in fishing as main occupation and about 400 families as part time occupation. The brackish water estuary systems of these panchayaths are extremely suitable for mussel culture. Six Self Help Groups of women (three each from both panchayaths) were selected as the sample and the data were gathered as explorative case studies through personal interviews of the respondents. For the study, the Group Dynamics of members of Self Help Groups was measured by developing an index called Group Dynamics Effectiveness Index (GDEI). Group Dynamics Effectiveness was operationally defined for the study as the sum-total of the forces among the member of SHG based on the sub-dimensions, such as participation, influence & styles of influence, decision making procedures, task functions, maintenance functions, group atmosphere, membership, feelings, norms, empathy, interpersonal trust and achievements of SHG. (Vipinkumar and Singh, 1998) For the computation of the Group Dynamics Effectiveness Index (GDEI), the scores obtained for each of the above mentioned sub-dimensions were first made uniform and then multiplied by the corresponding weightage assigned to each as by expert judges. These scores were then added up to get the GDEI score of each respondent. It was also ensured that all the sub-dimensions identified as components of GDE were of high significance on the basis of the coefficient of agreement in judges rating as well as the statistical evidence from the results of the pilot study. The measurement device developed for the dependent variable i.e., GDE was ascertained for its

content validity.

Measurement of sub-dimensions

A. Participation: For the present study, participation was operationally defined as the degree to which the farmer is involved in group meetings, discussions and group activities of SHG.

B. Influence & style of influence: Influence was operationally defined as the degree to which a farmer can influence other member of SHG in a desirable way. Style of influence was operationalised as the manner in which the member attempts to influence other members of SHG. The four different styles included were autocratic style, peacemaker style, laissez-faire style and democratic style.

C. Decision making procedures: This is operationally defined as the degree to which farmer makes a decision with involvement of other group member of SHG, makes decisions without topic drifting, supports other members' decisions in consensus, feels the majority's decisions valid in the SHG, attempts to get all members participate in decisions of SHG and feels the gains of recognition for his contribution in decision making process.

D. Task functions: This is operationalised as the degree to which the farmer makes suggestions to tackle a problem in the SHG, summarises what has been covered in the group, tries to give or ask for facts, ideas, opinions, feelings, feed back etc. and keeps the group on target.

E. Maintenance functions: This is operationalised as the extent to which farmer helps others into group activities of SHG, helps/interrupts him in group discussions, feels the other members are co-operative and listening, perceives other members help in clarifying the ideas of all members, feels good or bad when ideas are accepted or rejected and the extent to which other members attempt to maintain task functions of SHG.

F. Group Atmosphere: This is operationalised as the extent to which the group member prefers friendly congenial atmosphere in the SHG, attempts to suppress conflict or unpleasant feelings in the group, feels other members are involved and interested and feels satisfied from the work climate.

G. Membership: This is operationally defined

as the degree to which a group member feels accepted or included in the SHG, feels sub-grouping in the SHG and feels himself or other members to be outside the group.

H. Feelings: This is operationally defined as the degree to which the farmer feels anger/irritation, frustration, warmth, affection, excitement/boredom and competitiveness while performing the group activities of SHG.

I. Norms: This is operationalised as the extent to which the farmer feels the standards or ground rules and regulations are in operation that controls the behaviour of group members for the smooth functioning of the SHG.

J. Empathy: This is operationally defined as the degree to which the respondent is able to make out other person's feelings and thereby to understand it as he feels.

K. Interpersonal trust: This is operationally defined as the degree to which the respondent trusts the other members of the group as well as the faith other members have in him as perceived by the respondent.

L. Achievements of SHG: This is operationalised as the level of performance of SHG as perceived by the farmer as well as the performance of the farmer himself as the group member.

All these sub-dimensions were measured by a set of inventories containing appropriate questions arranged in a three-point continuum of always, sometimes and never with scoring pattern 2, 1 and 0 for positive and vice versa for negative questions. The cost estimates of all the selected Self help Groups were also computed and by taking in to consideration of major expenditure required for mussel farming is for the materials such as bamboo, nylon rope, coir, cloth, seed, etc. and labour costs essentially cover construction, seeding, harvesting etc. the Net Operating Profit and B:C ratio also were calculated for different SHGs to draw valid inferences. The basic data with regard to fisheries sector of Kasargod district is presented in Table 1. The study, focused attention on Group Dynamics Effectiveness as a trait of Self Help Groups resulted by the joint influence of individual members of the group generated out of skills and orientations from the past life experiences. It definitely varies from person to person, place to place, time to time, situation to situation

and in turn from group to group. This might be the probable reason for the differential degree of GDEI observed among respondents.

Table 1 General profile of fisheries sector in Kasargod district

Sl.No	Parameter	Kasargod
1	Length of the Coast line	70 km
2	No. of Marine Fishing villages	16
3	No. of Inland Fishing villages	2
4	Marine Fisherfolk population 2004-2005	45989
5	Active marine fishermen	10566
6	Inland Fisherfolk population 2004-2005	1004
7	Active inland fishermen	435
8	No. of Fisheries co-operatives	27
9	No. of domestic fish markets	164
10	Annual Marine Fish Production 2004-2005	8292 tonnes
11	Annual Inland Fish Production 2004-2005	1612 tonnes

Profile of Cost Estimates of Mussel Farming

The major expenditure required for mussel farming is for the materials such as bamboo, nylon rope, coir, cloth, seed, etc. and labour costs essentially cover construction, seeding, harvesting etc. The women's groups constituted in the scheme DWCRA started mussel farming as early as 1996-97 and are assisted by loan amount worth Rs 8800 / -per member with a subsidy amount worth Rs 4400/- which looks quiet fascinating. The duration of the loan is 5 years and the rate of interest is 12.5 % per annum. In addition to this, a revolving fund of Rs 5000 /- was also provided without interest. When the SHGs are economically empowered with the provision of loan facilities, the returns from mussel farming help them to

repay the loan slowly. The loan was granted through Farmers' Service Cooperative Banks and North Malabar Gramin Banks in Cheruvathur and Padanna panchayaths of Kasargod district. The expenditure details of the selected SHGs in the initial year of mussel cultivation are shown in the Table 2. The Net Operating Profit in all the six SHGs was computed and found as substantially good which proves the profitability of Mussel farming in the initial trial itself and since during the subsequent years, material costs such as those of bamboo, rope, cloth and labour cost in construction etc. are negligible, this ensures reasonable profit as a major consequence of adoption of Mussel farming enterprise bringing about economic empowerment of rural women through organized Self Help Groups.

Table 2: Cost estimates of the SHG's in mussel farming in Kasargod district.

	SHG 1	SHG 2	SHG 3	SHG 4	SHG 5	SHG 6
No. of ropes	500	800	600	750	900	725
Items						
Bamboo	6400	9600	7980	9000	11437	7800
Nylon rope	9954	17500	12000	15000	18000	14500
Coir rope	1100	1500	1200	1587	2000	1450
Cloth	3000	3250	1700	3338	3600	2250
Seed	6500	10000	8700	9000	10800	9770

Labour						
Construction	1600	2400	2170	2250	2700	2200
Seeding	1500	2565	1500	1875	2500	1800
Harvesting	1300	2000	1500	2000	2750	1875
Miscellaneous	1000	1600	1200	1500	1800	1450
Total Cost	32,354	50,415	37,950	45,550	55,587	43,095
Returns	40,000	64,000	48,000	60,000	72,000	58,000
Net Operating Profit	7,646	13,585	10,050	14,450	16,413	14,905
B : C Ratio	1.236	1.269	1.265	1.317	1.295	1.346
GDE Index	52.78	54.33	53.91	57.32	55.68	59.14

Experiences and observations already indicated that for a group to be developed as an SHG it requires a period of at least 36 months and it is a hectic process. It has to pass through various phases such as Formation phase, Stabilisation phase and Self Helping phase. These Self Help Groups promote a cooperative and participative culture among the members, which ensures the empowerment culture of the Self Helping phase. The loan sanctioning, utilisation, accounts maintenance and timely repayment of loans etc. are all perfectly accomplished with proper maintenance of the documented records by the group members. This ascertains the fulfillment of norms and standards of the SHG leading to economic empowerment of the members.

Open sea cage farming

Open sea cage farming is a promising venture which offers the fishers a chance for cultivating marine fishes and for optimally utilizing the existing water resources. As and R& D activity, CMFRI launched the first open sea cage 15 m diameter made High Density Poly Ethylene (HDPE) in the bay of Bengal off Visakhapatnam coast during May 2007. The second and third versions of marine cage were all found sea worthy at any extreme sea conditions. For easy management and cost effectiveness in terms of reduced labour, the size of the HDPE cages has been modified to 6 m in the 4th version. In a series of demonstration trials, these cages have been found to be successful in many maritime states along the Indian coasts. Latest version of pen sea cage is a cost effective GI cage designed for low investment farming operations found to be suitable in west coasts. Cage culture is a low impact farming practice with high economic returns. The system is eco-friendly

without any human intervention, and a higher survival of above 75% was achieved and sustained. The candidate fish species grown in cages are sea bass, red snapper, chanos, mullets, cobia, pompano, groupers, koth, pomfrets, lobsters etc. The mariculture in open sea cage devised under the present invention will expand a new mariculture space, thereby the mariculture scale can be expanded greatly; simultaneously the self-pollution of mariculture can be solved. Now a low cost cage made of GI pipes were are also being used in silent bays of east coasts. Self Help Groups initiated by CMFRI undertook cage farming for edible oyster in Moothakunnam areas.

Seaweed Culture

Around 60 species of commercially important seaweeds occur along the Indian coast from which, nearly 880 tonnes dry agarophytes and 3,600 tons dry alginophytes are exploited annually. CMFRI has developed technology to culture seaweeds by either vegetative propagation using fragments of seaweeds collected from natural beds or spores (tetraspores/ carpospores). Recently the culture of the carageenan yielding seaweed *Kappaphycus alvarezii* has become very popular and is being cultivated extensively along the Mandapam coast. The rate of production of *Gelidiella cerosa* from culture amounts to 5 tonnes dry weight/ ha while *Gracilaria edulis* and *Hypnea* production is about 15 tonnes dry weight/ha. Pilot scale field cultivation of *K. alvarezii* carried out in the near shore area of Palk Bay and Gulf of Mannar showed maximum increase in yield of 4.3 fold after 30-32 days in Palk Bay and 5.7 fold after 22-34 days in Gulf of Mannar. This is a promising venture being undertaken by the women's Self Help Groups in

Mandapam. So far as much as 1200 families are engaged in seaweed farming of which 60% of the farmers are women.

Conclusion

Mussel farming is achieving considerable significance because of its profitability. But it is inevitable to take care of the selection of suitable sites fulfilling the essential parameters for undertaking mussel culture trials. The consequence of adoption of mussel farming when accomplished through organized Self Help Groups of women in North Malabar areas is achieving considerable significance because of its tremendous profitability. Export potential of mussel can be promoted through value addition by depuration in filtered

seawater. Organised fishermen's cooperatives can play a vital role in various stages of seeding, harvesting, sorting, grading, packing and marketing with an intention of export potential. The study emphatically disclosed the deep rooted influence of Group Dynamics network among the farmer folk as influenced by their participation, influence & styles of influence, decision making procedures, task function, maintenance function, group atmosphere, membership, feelings, norms, empathy, interpersonal trust and achievements of SHG. Irrespective of the location specific problem oriented resource based alternative programmes for income generation, this study emphasizes on the economic empowerment of rural women through mussel farming through Self Help Groups.

Good practices in mariculture

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Introduction

The marine finfish in Asia are cultured in earthen ponds, floating cages or pen systems. The farming methods mostly involve stocking of fingerlings or juveniles that are caught from the wild or hatchery raised seed. Feeds used include trash or low-value fish, other fishery by-catch, farm-made feeds and commercial feeds. Marine finfish farming is not a well established activity in India. However, scope is there for its successful emergence in the immediate future because of the interest of the farmers in diversification of aquaculture species and the ongoing efforts in cage farming R&D and demonstration activities in the country. The high value species suitable for mariculture in India are Asian seabass *Lates calcarifer*, groupers *Epinephelus* sp., snappers *Lutjanus* spp., cobia *Rachycentron canadum*, silver pompano *Trachinotus blochii*, grey mullets *Mugil cephalus*, milkfish *Chanos chanos*, seer fish, pomfrets and a variety of marine ornamental fishes.

The term good practices (GP) have been used in several ways. It can refer to the best-known way to undertake any activity at a given time. In this sense, it probably refers to the practice or practices of only one or a very few producers. It can also be used to define a few, often different, practices that increase efficiency and productivity and/or reduce or mitigate impacts. Better practices are often required by government or others to encourage a minimum acceptable level of performance (and eliminate bad practices) with regard to a specific activity. In this sense, the term is used in opposition to unacceptable practices. GPs in aquaculture context have been used to outline norms for responsible farming of aquatic animals and plants. In aquaculture, good practices have been developed largely for shrimp and salmon aquaculture, although some efforts are being made to develop that for other aquatic commodities such as tilapias, catfish, molluscs, eels, etc, and marine cage culture.

GPs should involve positive cooperation rath-

er than more regulations. They are flexible and can be tailored to species, updated to fit new production methods.

Suggested GPs for sustainable mariculture with maximum production and minimum issues

1. Good site selection

- Based on current flow, wave action and wind speed, open sea sites can be selected. The site should be free from any fishing and other activities. To select ponds in coastal area specific criteria should be followed.
- Environmentally-sensitive areas that require an added degree of precaution owing to features and characteristics that support protected species and/or unique habitats (e.g., rearing or spawning habitat, migration corridors, protected areas or proposed protected areas, sensitive migratory bird habitat, etc.) should be excluded.

2. Design and construction of farms

- Eco friendly structures with minimum impact on environment should be used.

3. Planning of culture activities among fishermen/farmer community

- Plan farming activities in advance of the cropping season among the selected group(s)
- Plan the crop within the capacity of the group in terms of investment, level of commitment possible, and consider local water quality parameters and possible threats in that.
- Follow crop calendar system for farming (in such cases, the fishers will be occupied throughout the year and they will be provided with income during lean fishing season also)
- Implement all farming activities in a disciplined and cooperative manner.

4. Good quality seed selection (wild or hatchery reared)/ stocking density

- Select a high value species for which abundant seed availability is proven after proper survey.
- Quality of the seed and stocking rate (moderate stocking) of the fish in farms need to be standardized for individual species selected based on growth rate and feeding habits.

5. Water quality management

- Effluents of concern from pond aquaculture
- Nutrients (Nitrogen, Phosphorus)
- Settleable solids (especially from harvesting during draining)
- Oxygen demand from organic matter
- GP components to reduce effects of effluents from ponds
- Reduction of water exchange rates
- Reduction of production levels
- Use of feed trays
- Settling basins of at least 10% pond area
- Forcing farms to reduce discharge (only 2% water exchange rate)
- Stocking calendars to be prepared to follow a uniform pattern of stocking

6. Feeds and feed management

- Mostly high value marine fishes are carnivorous and are fed on trash fish/ bycatch. When trash fish is used as feed, it will not supply adequate nutrients to the growing fish. So for more growth excess feeding is done which results in increased FCR leading to high feeding cost and excess waste discharge to the ambient water (as uneaten feed and faecal matter).
- Select high quality feeds that contain adequate, but not excessive, nitrogen and phosphorous (to avoid eutrophication).
- Store feed in well-ventilated, dry bins, or if bagged, in a well ventilated, dry room. The feed should be used on a first in and first out basis by the expiration date suggested by the manufacturer.
- Apply feed uniformly
- Do not apply more feed than what fish will eat.
- Maintain adequate dissolved oxygen concentrations in ponds to prevent fish stress and en-

hance the capacity of the pond to assimilate metabolic wastes.

7. Health management

- Establish Fish Health Management Plan, a comprehensive plan for maintaining optimum health of the aquatic stocks in culture, usually consisting of procedures and guidelines for procuring healthy stocks, fish handling and transport, vaccination, feeding and veterinary practice.
- Regular monitoring of the cultured organism for health and growth

8. Disease management

- Make diagnosis for diseases and a recommendation for disease treatment before applying therapeutic agents. Disease diagnosis and recommendations for treatments should be done by fish health specialists.
- Therapeutic agents to be used only in closed systems. Manage pond water levels to prevent or minimize overflow until therapeutic agents have degraded. Use good water quality management procedures to prevent unnecessary stress to fish.
- Do not allow escape of infected animals to the main water source to prevent spread of disease to other farms

9. Better Harvest and post-harvest Practices

- Improve the quality and sale price of the crop by using better practices for crop harvesting and post-harvest handling of shrimp, fish and seaweed (which retain the freshness of the catch).
- Establish better market access by collaborating with a reliable and good local processor/ trader

10. Record maintenance of daily culture operation should be mandatory

11. Environmental awareness

- Fallowing or site fallowing, to discontinue production at a culture site for a short period, generally up to one season (or year) to sustain the environmental conditions
- People should be aware of Marine Protected Areas (MPAs), for the conservation and protec-

tion of: commercial and non-commercial fishery resources and their habitats; endangered or threatened marine species and their habitats; unique habitats; marine areas of high biodiversity or biological productivity; and any other marine resource or habitat for which special attention is needed should be exempted from aquaculture operations.

Suggested GPs for cage farming

- Cages should be placed in areas with good water circulation.
- It is ideal to change cage locations after each operation to protect the sediment quality.
- It is common for nets, cages, and other gears to become clogged or obstructed with natural foreign matter such as algal and invertebrate species. Deploy anti-fouling techniques to reduce the attraction of fouling organisms and/ or to remove them from the affected gear.

GPs for non-native species

- Measures must be taken to minimize the potential escape of non-native species, if they are stocked.
- Active feed monitoring as in closed systems
- Minimize uneaten feed accumulation beneath nets
- Proper disposal of feed bags
- Limit waste discharge during harvest & transport

Possible chances of fish kill in farms/cages and its management

Mortality due to disease or pathogen transmission from wild to farmed fish

- Licensed veterinarian to examine the cultured fish on a regular basis and treat as required.

Mortality due to predation

- Appropriate predator deterrence including predator nets, scaring devices, frequent removal of mortalities, regular inspection of nets.
- Mortality due to abrupt physico-chemical changes
- Select sites of suitable water temperature

Mortality due to hydrogen sulphide

- Do not allow farm waste to accumulate in the benthic environment.

Mortality due to algal blooms

- Consider the potential for algal blooms prior to site selection.
- Cages should not interfere with navigation or other permissible water uses.

Asian seabass (*Lates calcarifer*)

It is a high value carnivorous fish suitable for mariculture in Indian waters. The advantage of the species is that it can grow in very varied conditions and is tolerant to wide temperature ranges. The most advantageous fact is that the hatchery production of seabass seed is standardized and is being done commercially in India.

Contributions towards GP in mariculture by research institutes are:

1. Development of technical guidance documents for brackishwater/estuaries and coastal waters that will serve as "user manuals" for assessing trophic state and developing region-specific nutrient criteria to control over enrichment due to aquaculture practices.
2. Monitoring and evaluation of the effectiveness of nutrient management programs as they are implemented.

In shrimp aquaculture, well designed GPs can support producers to:

- increase efficiency and productivity by reducing the risk of shrimp health problems;
- reduce or mitigate the impacts of farming on the environment;
- improve food safety and quality of shrimp farm product; and
- improve the social benefits from shrimp farming and its social acceptability and sustainability

GPs could, in many instances improve the culture activities. Their impacts on resource use efficiency, productivity and more importantly on profitability, environment and social aspects can be similarly striking when compared to worse

practices. GPs can be country specific, or developed for a particular location, taking account of local farming systems, social and economic context, markets and environments. GPs are often voluntary practices, but can also be used as basis for local regulations, or even certification programmes.

Positive outcomes of GP in Indian aquaculture

- Decreased disease incidence:
- Increased confidence in contract hatchery system
- Reduced cost production: Through efficient use of feed (FCR of 1:1) and other resources, including reduced use of chemicals, all the farmers will achieve a very good profit for the first time in many years.
- Production of safe shrimp: No use of antibiotics. Seed, shrimp and other inputs have been screened for antibiotic residues and they were negative.
- Motivated farmers in abandoned areas:
- Cluster farm approach helps:
- To reduce the risk of disease outbreaks and improve the production in shrimp farms.
- To organize the farmers under "Self Help Groups"/"Aquaclubs" for sustainable production and to quickly meet the growing market demands.
- To produce better quality shrimps in socially acceptable, environmentally sound and economically viable manner.

Implementing GPs are done by creating:

- Awareness and capacity building of primary

producers

- Awareness and capacity building of other stakeholders in the supply chain
- Changing the attitude of key players
- Demonstrating the benefits of GP implementation

Approach followed can be by:

- Facilitation of collective approach (cluster farming)
- On-site programs to create awareness on GPs
- Assisting groups of aquaculturists to develop voluntary guidelines
- Facilitating participatory approach
- Providing regular technical assistance
- Linking to other stakeholders in the supply chain
- Monitoring compliance for adoption

Cluster Farming

Collective planning, decision making and implementation of crop activities by a group of farmers in a cluster through participatory approach in order to accomplish their common goal to reduce risks and maximize returns.

The dissemination of GPs can be done through:

- Farmers meetings
- Regular pond visits
- Extension material
- Brochures
- Booklets

List of Trainees

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5. Mr. Shahid Khalid
6. Mr. Ahmed Mauroof
7. Mr. Abdul Salaam Mohamed
8. Mr. Hassan Mohamed
9. Mr. Hassan Moosa
10. Mr. Ali Musthafa
11. Ms. Shafiya Naeem
12. Mr. Ali Nasir
13. Mr. Ali Nimaal
14. Ms. Nadhiya Yoosuf
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