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Training Manual on

HATCHERY PRODUCTION AND FARMING OF MILK FISH AND MULLETS

16th - 20th August, 2018

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14.08.2018

FOREWARD

The aquaculture sector bolsters food and nutritional security, employment opportunities and generates valuable foreign exchange for the country, hence, it is being increasingly recognised as a significant driver of the economic and social development of our nation in the days to come. We have deep roots in traditional and sustainable aquaculture systems and with needs of the time, have integrated scientific approach into our farming practices. Today, our shrimp farming sector contributes to the tune of 7.0 lakh metric tons during 2017-18 and is the economic phase of the Indian aquaculture scene. But, it is to be remembered that the sector was challenged by different pathogens related disease issues and crop failure at different points of time. The WSSV rattled the industry since nineties and the story of emerging diseases continues, with newly emerging diseases problems such as Enterocytozoon hepatopenaei (EHP), a parasitic disease difficult to eradicate. The changes witnessed have imparted crucial lessons on the need for diversified species including farmed finfishes for a holistic and sustainable aquaculture development in India.

ICAR-CIBA has foreseen the possibilities and promises of a sustainable brackishwater aquaculture sector based on multi-species farming as per the requirements of the different geographic locations of our country. CIBA as a nodal institute for Brackishwater aquaculture has played a pioneering role in brackishwater finfish diversification through R&D in developing technologies in captive reproduction, seed production and farming of major brackishwater species. The technologies developed by CIBA for Asian seabass, milkfish, pearlspot and brackishwater ornamental fish species vouch for our commitment to the same. The prime factors limiting the expansion and diversification of the different aquaculture species, the seed and feed requirements of the candidate species, are being duly addressed through the right scientific and technological interventions. CIBA has also taken a lead role in taking these technologies to the farmers and entrepreneurs, which reflected in the number of MOU's signed. We have technological capability to address the issues in terms of seed, feed, health and genetics.

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Kerala is blessed with abundant brackishwater resources, which needs to be brought into the fold of scientific aquaculture practices. The state is one of the most promising markets for finfish in our country, home to one of the most enterprising farmers and has rich nursery grounds for brackishwater finfish seeds. However, Kerala is lagging behind in taking up and propagating scientific principles in practicing Brackishwater aquaculture. CIBA recognises and supports the state fisheries government's proactive initiative in training its staff in the current technologies emerging in brackishwater aquaculture. I hope that the current training programme titled 'Hatchery production and farming of milkfish and grey mullet' aimed at imparting knowledge and skills on the recent technological advances in seed production and farming of the milkfish and grey mullet, will be useful to all concerned. I hope that it will also inspire the officials to develop brood-banks of the finfish and hatchery systems in different districts of Kerala after the completion of the training program

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

14/08

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PREFACE

Fish is a vital source of food which provides excellent nutritional value with high quality protein and vitamins. Aquaculture has emerged as fast growing food producing activities globally, which supports not only nutritional security but also contributed towards employment generation and earning of foreign exchange. Maintenance of sustainable growth in this sector is primarily depends on the culture of diversified finfish and shellfish species instead of depending fewer species. Therefore, species diversification is very essential for sustainability since the varying nature of brackishwater bodies in India require different finfish species with respect to its adaptation to different salinity regime, feeding habits, growth rate etc., to take up large scale farming practices. ICAR-Central Institute of Brackishwater Aquaculture has developed a comprehensive technology package for seed production of Asian Seabass, milkfish and Pearlspot. CIBA has taken lead role in standardizing broodstock development under captivity, breeding protocols for grey mullet and brackishwater ornamental fishes. CIBA has signed MoU with farmers and entrepreneurs to demonstrate the seed production, farming and feed development for commercially important brackishwater finfishes in partnership mode so as promote the adoption of culture practices by the farmers.

As requested by the Department of Fisheries, Govt. of Kerala, the training programme is being conducted at CIBA, Chennai for five days from 16th to 20th August 2018 to provide hands on training to the officials of DoF, Govt. of Kerala on "Hatchery Production and Farming of Milkfish and Mullets". The presentations of the lectures are based on the field experience and knowledge gained by the distinguished scientists working in their specialized fields. We hope that this training manual would be very useful to the particpants involved in brackishwater aquaculture. In this context, I would like to express my heartfelt gratitude to Dr. K.K.Vijayan, Director, CIBA for the guidance and support rendered to organize this training course. I am very grateful to my colleagues for their continuous efforts for making all the arrangements well as for compilation of the training manual. I sincerely thank all the faculty members for their timely submission of the lecture notes. The support and involvements of technical officers, supporting staff of FCD administration and finance for this training programme is gratefully acknowledged.

14th August 2018

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RECENT ADVANCES OF BRACKISHWATER FINFISH AQUACULTURE IN INDIA- MILESTONES AND THE WAY AHEAD

K.K. Vijayan, M. Kailasam

Introduction

The brackishwater aquaculture sector is a significant contributor to the economic development of our country by earning valuable foreign exchange, providing direct and indirect employment, livelihood opportunities and ensuring nutritional security. Indian aquaculture sector has witnessed a 6.5 fold increase in the past two decades and contributed 4.8 million tonnes to the total fish production of 9.8 million tonnes in the year 2014 (FAO, 2016). India is endowed with vast areas of brackishwater aquaculture resources amounting to 1.2 million ha, thisis beside the 1.24 million ha salt affected soils in coastal areas of about 6.1 million ha inland saline soils, only a fraction of which is currently utilised (over 1.15 lakh ha). These figures highlight the potential and scope for the development of brackishwater aquaculture in our country.

Brackishwater aquaculture has been an age old practice in the traditional culture systems of India in the form of traditional prawn filtration systems *Pokkali fields* of Kerala, the *bheris* of West-Bengal, *Gheris* of Orissa, *Khar* lands of Karnataka and *Ghazni* fields of Goa. As early as 1911 James Hornell suggested the development of salt water fish farming in Madras Presidency which led to establishment of marine fish farm near Tuticorin by utilising few lagoons in the area and stocking mullets and sand whiting (*Mugil spp.* and *Sillago sp.*). Establishment of Narrakal fish farm at Kochi during 1940-42 symbolised the initiation of brackishwater fish farming (mullets and milk fish) on a larger scale. Similarly Ayiramthengu fish farm adjoining Kayamkulam lake in Kerala was a significant initial step in the development of the culture of brackishwater fish (pearlspot, mullets and milkfish) (Tampi 1958). Another major milestone in the development of brackishwater aquaculture in our country was the initiation of All India Coordinated Project (AICRP) on brackishwater fish farming by ICAR in 1973 resulting in the development of many finfish and shrimp farming technologies.

Despite all these efforts brackishwater aquaculture has been largely synonymous with shrimp aquaculture owing to the high export potential of shrimps. (Rao and Ravichandran, 2001). The early 1990's was characterised by a boom in unregulated shrimp farming activities dominated by the giant tiger shrimp *Penaeus monodon.* This however faced a set-back due to the emergence white spot disease (WSD) caused by white spot syndrome virus (WSSV). Since 1993 WSD has continued to pose a major challenge to the shrimp farming. The consequent economic losses continues to affect the farmers and the shrimp farming sector even today. This has led to the introduction of exotic SPF *Penaeus vannamei* in 2009-10, and in a matter of 5 years, we witnessed a changed scenario of the shrimp farming sector being dominated by white legged shrimp (*P. vannamei*), which has totally replaced the Indian tiger shrimp, a species cultured in 1990's to 2010. This major species shift took place after a cautious risk analysis undertaken by the government with ICAR-CIBA playing a key role in the introduction of this fast growing species with high export potential. Today again as we take stringent steps to address the potential threat posed by EHP to the shrimp farming sector, we are also vociferous in promoting diversification of aquaculture species and practices. ICAR-CIBA has has believed that species diversification in aquaculture and developing need based location specific technologies is the best roadmap for sustainable aquaculture sector for our country.

The development of brackishwater finfish seed production technology in India

The prime brackishwater finfish species having high consumer preference and market demand in India include Asian seabass *Lates calcarifer* (Bloch, 1790), grey mullet *Mugil cephalus* (Linnaeus, 1758), milkfish *Chanos chanos* (Forsskal, 1775), pearlspot *Etroplus suratensis* (Bloch 1790), and red snapper *Lutjanus argentimuaculatus*. In the year 1997, a significant milestone achieved with respect to brackishwater finfish aquaculture in our country ~~was~~ with the successful breeding of Asian seabass in captivity, at the Central Institute of Brackishwater Aquaculture. The R&D in seabass breeding thus led to the establishment of the first brackishwater/ marine finfish hatchery of our country located at MES, CIBA, Chennai.

After the successful induced breeding of freshwater carps in 1950's, it was after a spell of four decades that a breakthrough was achieved in successful induced breeding and larval production of seabass, a brackishwater/ marine foodfish in our country. Subsequently seabass hatching and rearing technology, developed by CIBA was transferred to Rajiv Gandhi Centre for Aquaculture (RGCA) during 1999-2000 (CIBA Annual Report 2000-01). The hatchery produced seeds are being produced and supplied to farmers, which is further spreading the seabass farming in country. Today private entrepreneurs are taking interest in the farming of seabass, and the demand for hatchery produced seeds of finfish species is on the rise, reflecting the increasing interest of brackishwater farmers in adopting seabass nursery rearing and farming technology. Significant progressive efforts towards the breeding of other candidate species such as cobia *Rachycentron canadum* (Linnaeus, 1766) and silver pompano *Trachinotus blochii* have been made in our country by institutions such as Central Marine Fisheries research Institute (CMFRI), Central Institute of Brackishwater Aquaculture (CIBA) and Rajiv Gandhi Centre for Aquaculture (RGCA). For these species the hatchery based seed production and aquaculture are in their formative stages.

The declaration of pearlspot, "Karimeen" as the state fish of Kerala in 2010 came as a boon to this indigenous cichlid which has been an important brackishwater food fish of Kerala, with great local demand with a market price in the range of Rs. 300 to 600/kg. The policy helped to lay a new focus on the species leading to the revival the aquaculture for improving the overall pearlspot production in the state. The year 2015 will go down in the history of Indian brackishwater aquaculture for another significant milestone achieved- the first successful captive breeding of the marine herbivorous fish species, the milkfish *Chanos chanos* by ICAR-CIBA. The herbivorous species forms the mainstay of brackishwater finfish aquaculture of many south-east Asian countries such as Indonesia and Philippines. Farming of milkfish can play a significant role in the development of sustainable models of brackishwater aquaculture in India, in the days to come. In the year 2016, the captive breeding of milkfish by CIBA was followed up by successful larval rearing and fry/ fingerling production and its distribution to farmers for growout culture, for the first time. Successful results came from the efforts for transporting the batches of fertilized eggs of milkfish to private entrepreneur and the subsequent rearing of milkfish larvae to fry stage and its sale to growout farmers. Grey mullet *Mugil cephalus* by virtue of occupying lower trophic levels of the food chain and at the same time having a high market value is a species of significance for sustainable aquaculture systems like the IMTA (Integrated Multi-trophic Aquaculture) and finfish polyculture. We are still reliant on wild seed resources for its aquaculture the availability of which is today getting limited due to deteriorating nursing grounds and emerging regulations on wild seed collection by states like Kerala. Hence ICAR-CIBA has laid high impetus on developing a technology for grey mullet captive seed production.

Brackishwater ornamental aquariculture is also given a special focus and being developed as new area on account of its potential to play a significant role in providing livelihood to small scale farmers and Self Help Group's, by adopting the homestead rearing model. CIBA has bred spotted scat, *Scatophagus argus,* Moon fish *Monodactylus argenteus* for the first time in the country. Species such as orange chromide, crescent perch, pearlspot are also being developed as ornamental fish production models, suitable to get regular monthly incomes to farmers. CIBA has been successful in breeding and successfully developing farming models of the catfish *Mystus gulio* which commands good market value in the states such as Bengal.

Brackishwater Finfish aquaculture- The Present Scenario

Asian seabass has been mooted as a prime candidate for diversification to finfishes on account of its high market demand (Rs.300-400 per kg), and availability of hatchery produced seed and formulated extruded feeds. CIBA has achieved an average larval survival rate of 30% and a maximum survival of 62% (Arasu et al., 2012) which is at par with global standards. The global Asian seabass production from aquaculture was to the tune of 71,851 t in 2014. Globally seabass is cultured in different culture systems like cage, ponds, pens and tanks showing the adaptability of the fish to the different culture methods. In India seabass is primarily cultured in brackishwater pond systems where it exhibits growth rates ranging from 350 g- 1.5 kg, in a period of 6 month to 1.2 years. Partial harvesting of larger fish is carried out periodically in this extensive farming practice. Monoculture of seabass is practiced at stocking densities of 10000-20000 ha^{-1} (initial wt.-2.0 g) and a production of 2.5-5 t ha^{-1} with a survival of 30-70 % is observed in 10 months. The cost of production is around Rs. 175-225/kg at a fish sale price of Rs 350-400. To develop a holistic model of seabass culture, a formulated seabass feed, 'Seabass Plus' has been developed by CIBA for nursery and growout culture which gives an FCR of 1.5-1.7 and the feed cost is approximately Rs. 55/kg. Presently, seabass is considered as one of the most potential finfish aquaculture candidates for those looking for diversification from shrimp culture.

Herbivorous species form the backbone of sustainable fish farming models due to the lower cost of production- milkfish, grey mullets and pearlspot have been traditionally cultured by enhancing the ponds natural productivity and through low cost supplementary feeding using agro-by-products. CIBA's effort on the seed production of these fish species bore fruit with the captive breeding of milkfish at its Muttukadu Experimental Station (MES). Today hatchery produced milkfish seeds distributed to brackishwater farmers across the country are being cultured using growout formulated feeds specially developed for milkfish. Interventions in parental care of pearlspot has assured enhanced seed production and given rise to a modular tank based system for seed production for adoption by small scale farmers.

Few models of finfish culture being given key focus by CIBA

In keeping with its slogan "Brackishwater aquaculture for food employment and prosperity", icar-CIBA recognizes that brackishwater aquaculture is a powerful tool to bolster livelihood and nutritional security. Hence, focus has been placed by CIBA to develop location specific need based models for different stakeholders.

i) **Satellite based nursery rearing of seabass** - One of the interesting innovative approaches adopted by CIBA for developing seabass as a separate livelihood activity is the hapa based nursery rearing model where hatchery reared fry are cultured for a duration of about 45 days to fingerlings size. This model is being mooted both as a livelihood activity for farmers for giving returns in a short duration and also for developing satellite seabass seed rearing centres to facilitate widespread adoption of seabass aquaculture. This helps in saving space and time during the grow-out culture of seabass and helps farmers to tide over the phase which requires physical labour for frequent size grading of the fry. Adoption of nursery rearing of seabass fry by farmers involved in low volume cage culture of Asian seabass *Lates calcarifer* is encouraged by ICAR-CIBA. The activity helps farmers get a better control over the initial size used for stocking in cages, a factor critical for getting optimum survival rates and fish production. A farmer having a 1 ha pond, can stock 50,000 numbers of seabass fry in 50 hapas (2x1x1m). Considering a seed cost of Rs 2,50,000 (Rs 5 per unit), feed cost- Rs 50,000, installations and labor Rs 1 lakh and a final survival rate of 70 %, amounting to a revenue of Rs 5.25 lakh (fingerling sale price Rs 15 per unit) , a benefit cost ratio of 1.31 is worked out for the seabass nursery rearing activity.

ii) **Low volume cage culture** - The access of small scale farmers to diverse opportunities offered by different aquaculture initiatives is often limited by ownership or access to water resources, access to simple and adoptable technology and high investment costs. Production of high value fish using low volume cages set in brackishwater bodies can thus be a potential livelihood option to the poor. Low volume cages can be fabricated by the farmers themselves. Species like seabass stocked at rates of 30-40 fry per cubic-m has shown to yield a production upto $20 kgm^{-3}$. For example, in a demonstration using 3 cage units of 8 m^3 each, a production between 450-500 kg of seabass was obtained Partial harvest of the fish from the cages can thus provide the family with sustained monthly income of Rs 10,000-15,000 by sale of the fish at Rs 400/kg. Construction and setting up of these low volume cages is also being mooted as a skill development activity for small farmers.

iii) **Integrated Multi-Trophic Aquaculture (IMTA)** - IMTA is the farming of aquaculture species from different trophic levels and with complimentary ecosystem function. For laying a roadmap for sustainable aquaculture, CIBA is developing economical polyculture models and adopting IMTA (Integrated Multi-Trophic Aquaculture) approach. These models have been successfully demonstrated at Kakwdip, West Bengal and Sindhudurg, Maharastra with farmers participation. The benefit cost ratio of pond based IMTA was worked out to be 1.5 as compared to 1.4 in monoculture of shrimps at the culture demonstrated at Sindhudurg district of Maharastra. Culture demonstrations at Kakwdip centre of ICAR-CIBA has shown the environmental and economical benefits of IMTA over conventional culture practices.

iv) **Zero stocking models** - CIBA also realises the relatively longer culture duration of finfish compared to shrimps as a possible stumbling block for adoption of finfish aquaculture. For this, CIBA advocates adoption of a multi-phased model for finfish culture. A model has already been tested with seabass where the long culture duration has been split into three phases- the nursery, pre-growout and growout phase.

v) **Finfish seed production from egg stage** – Realising the need for elaborate investment and infrastructure for maintenance of broodstocks of finfishes, private entrepreneurs are encouraged to transport fertilized eggs for hatching and subsequent larval rearing in their hatcheries. This model has been successfully adopted in case of candidate species like seabass and milkfish by private entrepreneurs.

vi) **Ornamental fish seed rearing as a household activity** - CIBA is mooting the adoption of nursery rearing of ornamental fish like orange chromide, pearlspot, and crescent perch as an activity to be adopted at household levels for getting regular monthly income. As a part of the Mera gaon mera Gaurav program of CIBA, pearlspot nursery rearing as alivelihood activity is being successfully adopted by tribal women groups.

Way forward for brackishwater finfish aquaculture development and CIBA's interventions

For the development of brackishwater finfish aquaculture, mass availability of hatchery produced seed during at the right time in the required quantity continues to be the single major constraint. For this CIBA has placed a major thrust on developing economically viable seed production technologies of the prime brackishwater candidate species such as seabass, cobia milkfish and grey mullet. The development of hatchery technologies are also being followed by partnership with entrepreneurs for facilitating technology adoption in different states in PPP mode, this is witnessed in the partnerships both for shrimp and finfish species. There is a need of private sector hatcheries for the scaling up of seabass seed production for catering to the increasing demand of seabass and milkfish fry among brackishwater fish farmers.

For the development of large scale economical finfish aquaculture practices, development of efficient, eco-friendly and low cost feeds is perceived as the next major challenge. The problem has aggregated with our dependence on fishmeal as a primary protein source in feeds. CIBA has developed feeds for Asian seabass and other major species are being tested. By entering into partnerships CIBA is also facilitating the development of feed mills and developing feeds for different brackishwater foodfish and ornamental species.

Considering the significance of aquaculture as a tool to alleviate poverty, provide livelihood and nutritional security, CIBA lays a major thrust on developing family farming models for widespread adoption in different states. Thrust is also being laid for developing suitable marketing models for getting the best price for the farmer.

CIBA visualises and strives for a holistic sustainable development of the brackishwater aquaculture sector of India with an underlying thrust on sustainability, economic viability and livelihood provision. For this CIBA reaches out for active partnerships with the state government, the private sector, other research organisations and Self-Help-Groups to develop and advance the brackishwater aquaculture technologies for the betterment of the Indian brackishwater aquaculture sector.

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AN OVERVIEW OF INDUCED BREEDING TECHNIQUES OF BRACKISHWATER FINFISHES

M. Kailasam, S.N.Sethi, G.Biswas, K. Sukumaran, Prem Kumar, Aritra Bera, Babita Mandal

Introduction

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

Selection of breeders

Breeders can be obtained either from wild or from broodstock developed in captivity. One of the problems faced in induced breeding is that variations occur in the gonadal development among individual fish both in the wild and in broodstock developed in captivity. Successful induced breeding depends upon the selection of the recipient fish at the proper stage of the gonad development. Normally, the external characters like fullness of belly, colour and state of swelling of genital opening such as protruding pinkish/reddish, genital papilla, softness and resilence of the belly (in females), roughness of pectoral fins, presence of hard tubercles (in males) etc. were considered for the selection of breeders. However, many of these parameters are not absolutely reliable. For example, enlargement of belly can be due to presence of food in the intestine and stomach. The more reliable method to assess the maturity of females now being used is through ovarian biopsy taking a sample of the ova using a catheter and to examine them under microscope. The mature ova will have round shape and non-adhesive. The average ova diameter has to be determined and this is used as an important criterion in the selection of females for induced spawning. In the case of males, maturity is ascertained by applying pressure on either side of the belly. In the case of fish in mature condition, milt will be flowing through the genital opening on application of gentle pressure.

Sex determination

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

Methods of Breeding

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

Artificial fertilization by striping

In this method spawners are obtained from wild during the natural breeding season. In seabass breeding is related to lunar cycle. Again breeding occurs before midnight during high tide. Even though the fish breeds both during the new moon and full moon phases, quality of eggs released during full moon phase is better and the number of eggs released also will be more. Fishes caught during full moon and new moon phases and during high tide are examined for maturity. Both males and females that are in oozing stage can be striped and fertilized artificially. In oozing females the diameter of the eggs will be around 0.7 to 0.8 mm with large oil globule. The eggs will be almost transparent. The ripe eggs will scatter individually whereas unripe eggs tend to group together in water. In water having salinity 28 – 30 ppt the ripe eggs will float.

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

Environmental manipulation

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

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

Induced Spawning

Seabass does not spawn in the broodstock tanks normally. Administration of reproductive hormones becomes necessary for inducing them to spawn. Human chorionic Gonadotropin (HCG), Puberogen, Pregnyl and Luteinizing hormone – releasing hormone analogue (LHRH-a) are the main reliable synthetic hormones that are used for induced breeding.

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

At Central Institute of Brackishwater Aquaculture, Chennai, des – Gly 10 (D-Ala 6) luteinizing hormone releasing hormone ethylamide acetate salt (LHRH-A) hormone is used for the induced breeding of seabass. Breeding is normally taken up on new moon or full moon nights. Female and male breeders are selected in the ratio 1:2 in the broodstock tanks and transferred to the hatchery. Their total length and weight are recorded and also ascertained that they are in good health condition. LHRH-A is administered to females and males @ 60 – 70 ug/kg body weight and 30 – 40 ug/kg body weight respectively and transferred to the spawning tank. Water salinity 30 – 32 ppt was found to be optimum for spawning. The breeders should be free from disturbances like excess noise and human movements. They spawn after 30 – 36 hrs of hormone administration. The spawning may continue for a week releasing 3 – 4 batches of eggs.

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

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

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

BIOLOGY OF MILKFISH (*CHANOS CHANOS*)

Babita Mandal, Aritra Bera, ,Tanveer Hussain, T. Sivaramakrishnan

Introduction:

Milkfish *Chanos chanos*) is one of the most popular cultivable brackishwater finfishes in the south east Asian countries and widely distributed in the Indo-Pacific region. The maximum weight and age of this fish were reported as 14kg and 15 years respectively. In India, it is named as *Paal Meen* in Tamil, *Pala Bontha and Tulli Chepa* in Telugu, *Poomeen* in Malayalam, *Hoomeenu* in Kannada, *Golsi* in Goa and *Seba khainga* in Oriya. Being herbivore, milkfish feeds on plankton, benthic algae, detritus matter in the natural condition and easily accepts the pellet feed under culture condition. It can tolerate and live in extreme salinity ranging from 0-100 ppt but growth is optimal between 0.5-40 ppt. Milkfish can attain the table size weight from 400 to 500 gm in 5-6 months under culture condition. Milkfish having tiny bones resemble with Hilsa and can be considered as a '*Decan Hilsa*'. Milkfish can be produced in the farm with the production cost of Rs.80-90/kg by feeding with low protein pellet feed.

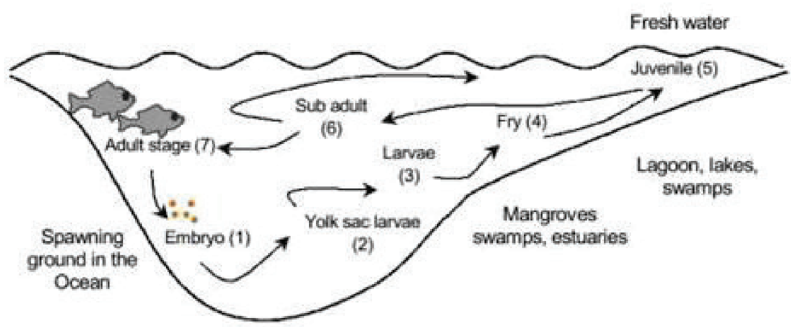
Kingdom – *Animalia*
Phylum – *Chordata*
Order – *Gonorynchiformes*
Family - *Chanidae*
Genus – *Chanos*
Species - *Chanos chanos*

Adult milkfish (50-150 cm total length) are powerful swimmers and mostly found in open sea. During the breeding, it migrates towards reefs and sandy-rocky shores for breeding. Milkfish fertilized eggs appear slightly yellowish (size 1.10 - 1.25 mm). Incubation period is ranged 24-26 h. Milkfish fry are most abundant in shore waters. Large juveniles and sub-adults return to sea. During 1986, the Southeast Asian Fisheries Development Center (SEAFDEC) at Phillipines under National Bangus Breeding Program successfully established milkfish seed production technology. Global production of milkfish is estimated to be 9 lakh metric ton during 2012 Philippines is the leading producer of milkfish with 391,983 MT during 2014 apart from Indonesia and Taiwan.

Wild milkfish seeds are collected during March to May from Andhra Pradesh and Tamil Nadu coasts by traditional methods and farmed in the coastal ponds along with other finfish and shell fishes. ICAR-CIBA has made major breakthrough on captive breeding of milkfish for the first time in India during June 2015 and developed comprehensive technology package for seed production of milkfish. Captive breeding of milkfish involves development of land based captive broodstocks (6+ years old) and application of calculated dose of slow release hormone (LHRH-A) pellet. Hatchery produced seeds were distributed among farmers for promotion and demonstration of milkfish farming in coastal states. To educate the farmer community about this technology, routine trainings are being organized by ICAR-CIBA on need-based.

Life history and habitat

Adult milkfish (50-150 cm total length) are found in open sea, swift and powerful swimmers. During the breeding season, they migrate towards coasts where reefs and sandy-rocky shores for breeding. Spawning takes place in the open sea and the eggs are pelagic. Milkfish fertilized eggs appear slightly yellowish (size 1.10 - 1.25 mm). Eggs float at 34 ppt and tend to sink at <30 ppt salinity. Incubation period is ranged 20-35 h at temperatures of 26-32°C and salinities 29.5-34 ppt. newly hatched larvae measure 3.5 mm TL at hatching, have a large yolk sac (volume, 0.5 µl), unpigmented eyes, and no mouth. They grow to about 5 mm in 36 h after consuming about 90% of the yolk. Milkfish fry of 10-17 mm are most abundant in shore waters. The larvae and juveniles spend their lives in inshore estuarine areas and then migrate into rivers in the direction of fresh water (Lin et al., 2003; Pillay and Kutty, 2005) for further growth. Milkfish juveniles larger than 20 mm have the characteristic shape and morphology of the adult fish.



Source: Life cycle and habitat of milkfish in wild **(**Martinez *et al*., 2006**)**

Distribution worldwide

Milkfish can tolerate wide ranges of salinity and temperature fluctuations (Gordon and Hong, 1986). Milkfish occurs near coasts and islands in the tropical Indo-Pacific area and is centered around the Philippines and Indonesia. It inhabits areas of the Pacific Ocean which is affected by warm ocean currents. Their distribution can be found near to coral reef areas where the water is clear, shallow and warm (more than 20°C). Milkfish has been recorded in the Red Sea, off the coast of East Africa, including Zanzibar and freshwater lakes in Madagascar, Mexico, coastal parts of India, Sri Lanka, Malaysia, Vietnam and Thailand. Milkfish is abundant in Kiribati, Fiji, Tonga, and New Caledonia. In Kiribati milkfish has been produced mainly as bait fish for supporting the tuna fishing industry.

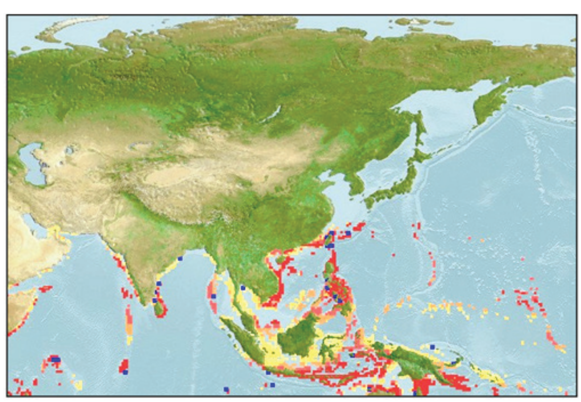
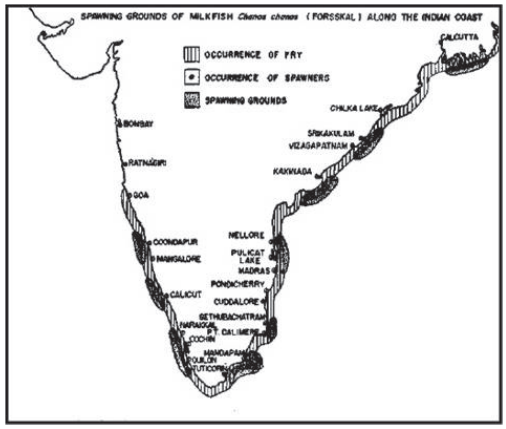


Fig: Worldwide Milkfish Distribution

Distribution in India

Initial observations on the fry of milkfish in India were made by Panikkar et al. in 1952. Adult milkfish from has been reported from CoroMandal Coast, Palk Bay, Gulf of Mannar andMalabar Coasts (Chacko and

Thomas, 1962), Salt pans of Kakinada (Dwivedi and Reddy, 1977), Pulicat lake (Rao, 1970), Vishakhapatnam, Krusadai and Rameswarm Islands (Chacko and Mahadevan, 1956), Bakkhali region of lower Sunderbans (Basu and Prasad, 1976). On the basis of occurrence of adult milkfish, spawning ground has been classified as Madras, Mandapam, Tuticorin, Vizhinjam, Cochin, Calicut zone (Silas et al., 1985). Estuaries and mangroves areas were recorded with abundance of fry after spawning season.



Source: Proceedings of the symposium on coastal aquaculture (Silas et al., 1985).

Food and feeding habits

The morphology of the digestive system of adult and juvenile milkfish suggests that it is mainly an herbivore (Chandy and George, 1960; Kinoshita, 1981). It has a toothless mouth with fine closely laid gill rakers and a pair of muscular rakerlined with epibranchial organs. The esophagus is long and thick-walled. The stomach is large and the cardiac region is characteristically bent (doubled-over). The pyloric region has a spherical gizzard with a mucus membrane and very thick walls. The cardiac stomach has gastric glands. The gizzard seems to function in trituration of coarse food materials. Numerous pyloric caeca clusters can be found behind the gizzard. The intestine is extremely long and convoluted. Adult milkfish can be kept in captivity on a diet of commercial pellets with about 42% protein given at 1.5-2% of body weight twice daily (Marte and Lacanilao, 1986). After full pigmentation of milkfish fry eye, mouth opens at 54 Hour after hatching. The yolk is completely adsorbed within 120 hour post hatching. After three days of hatching, esophagus and intestine can be differentiated. Mouth size is 200 µm, after opening the mouth of milkfish larvae (225 µm body sizes). Copepods and Diatoms are the main feed for milkfish fry in coastal waters. Larvae are visual feeders. In hatcheries larvae are fed with rotifer and *Artemia* nauplii and later weaned on artificial larval diets. Milkfish fry cannot digest rigid cell wall of chlorella in juvenile stage. After 2 weeks, Non-live feed can be given to larvae. 40% weaning can be achieved with finely ground artificial diets at juvenile stage. In nature, juvenile milkfish is a bottom feeder (iliophagous). It ingests the top layer of bottom sediments. Digestibility of fish meal and soybean meal is lower in seawater as compare to freshwater. Therefore, food conversion efficiency in milkfish got decrease and protein requirements increase in seawater conditions. Being an opportunistic filter feeder, adult milkfish mainly feed on zooplankton, larval and juvenile clupeiods in wild.

In artificial intensive culture systems, milkfish require 30 % or more protein and a lipid level of not less than 7%. Milkfish broodstock requires 1000 mg Vit. C/kg feed for good egg and larval quality. Adult milkfish accepts a variety of diets like sinking particles and floating pellets. Use of pellets improves the feeding efficiency. Pellets are having physical characteristics such as better stability which prevents feed particles from dissolving and leeching of nutrients in the water. Adult fishes are fed with pellets of approximately 4-5 mm (dia) and 6-8 mm long.

Growth

However, growth rates in ponds and in pens vary considerably depending on initial fish size, food, stocking density, climate, season, locality, water turnover rate, pond area and depth, pests and predators. The growth curve of milkfish larvae is sigmoid. Some reports have found that hatchery-bred and reared milkfish fry are generally heavier and morphologically more advanced (heavy pigmentation, pelvic fins present) than shore-caught fry of similar length. Growth rates of wild and pondreared juvenile milkfish vary from 7.0 to 8.7 mm weekly.

Growth and Reproduction:

Larval stages of Milkfish exhibit sigmoid growth curve. Hatchery-reared milkfish fry tend to be growing fast and morphologically more advanced than wild caught fry of similar length. Adult Milkfish grows very well in ponds, compared to other cultured finfish and shellfish species. Growth rates in ponds and in pens vary considerably depending on initial fish size, food, stocking density, climate, season, locality, water turnover rate, pond area and depth, pests and predators. Sexes are separate in milkfish but morphologically identification is difficult. Ripe male oozes milt when pressure is applied to the abdomen. In case of gravid female fishes, abdomen may appear distended. Mature ovary of female fish grows 10 – 25 % of body cavity. Milkfish do not spawn in freshwater. Milkfish have been observed to spawn at water temperatures of 24-33°C. In nature, spawning of milkfish has been observed during the first quarter and full moon periods. Peak season of spawning lies during March – June which again varies according to geographic regions. A 5-13 kg female can produce 3, 00,000 – 5, 00,000 eggs/kg body weight.

Larval Stages

Milkfish larvae are constituents of open sea plankton. Newly hatched larvae measure 3.5 mm TL at hatching, have a large yolk sac (volume, 0.5 ml), unpigmented eyes, and no mouth. They grow to about 5 mm in 36 h, consuming about 90% of the yolk, and grow very little until day 5 when the yolk is completely exhausted . Egg size, larval size, amount of yolk, and mouth size are greater in milkfish than in many other tropical marine fishes (such as the sea bass, *Lates calcarifer* and the rabbitfish, *Siganus guttatus.* This size advantage is probably one reason for the relative ease in rearing milkfish larvae in the hatchery and for the abundance of milkfish fry in the wild. Total lengths are given for larvae from the plankton measured in the preserved condition and for larvae reared in the laboratory and measured in the fresh state (in parentheses).

Fry and the Transformation Stage

Milkfish fry are most abundant in shore waters and constitute a valuable fishery that supports the centuries-old pond culture industry in southeast Asia and India. Fry caught in shore waters are all within a narrow range of 10-17 mm TL. The transformation stage, also termed the “metamorphic stage,” is a complex of morphological developments accompanied by many physiological and behavioral changes

Juveniles

Milkfish larger than 20 mm acquire the characteristic shape and morphology of the adult of the species and are considered juveniles. Juveniles <10-cm long arc usually called fingerlings. They bear complete fin-ray complements, forked caudal fin, scales, and silvery coloration. Juvenile milkfish have been found in such diverse habitats as coral lagoons, mangrove lagoons, estuaries, marsh flats, tidal creeks, and tide pools that share the common characteristics of rich food deposits and protected, relatively shallow waters

BIOLOGY OF GREY MULLET (*MUGIL CEPHALUS*)

Rekha M.U., Krishna Sukumaran, Pankaj Patil, Dani Thomas, Tanveer Hussain

Introduction

Mugil cephalus L. is cosmopolitan and contribute significantly to the economy of countries of Southeast Asia, Mediteranean region, Taiwan, Japan and Hawaii. This species is euryhaline and capable of surviving in wide variety of marine, estuarine and freshwater environments of varying turbidity, salinity and dissolved oxygen levels (Thomson 1955, Ibanez and Guitierrez-Benitez 2004).

Taxonomy of *Mugil cephalus*

Kingdom	:	Animalia
Phylum	:	Chordata
Superclass	:	Actinopterygii
Class	:	Teleostei
Order	:	Mugiliformes
Family	:	Mugilidae
Genus	**:**	**Mugil (Linnaeus, 1758)**
Species	**:**	***Mugil cephalus***
Common Name	:	Striped mullet, grey mullet, Flathead Mullet

Morphology of *Mugil Cephalus*

Body is cylindrical and robust. Head is broad with its width more than width of mouth cleft. The labial teeth of upper jaw are small, straight and dense, usually in several rows. The mouth cleft ending below posterior nostril. The fish has two dorsal fins; the first with 4 spines; the second with 8-9 soft rays; origin of first dorsal fin nearer to snout tip than to caudal fin base; origin of second dorsal fin at vertical between a quarter and a half along anal fin base. Anal fin with 8 soft fin rays. Pectoral fins with 16-19 rays. Scales in lateral series range from 36-45.

Body Coloration

The body colour is grayish olive to grayish brown, with olive-green or bluish shades and sides fading to silvery white towards the belly. Dark longitudinal lines, formed by dark spots at the center of each scale on the upper half of the body, run the length of the body. Young fish smaller than 6 inches (15 cm) in length lack stripes. A large dark blotch is at the base of the pectoral fin.

Distribution in India

M. cephalus comes under family Mugilidae, which comprises a total of 20 genera and 70 valid species (11 of which belong to the genus *Mugil*) (Eschmeyer and Fricke 2011). In India 13 species of mullets are well recognised. Of these, 8 species contribute to the commercial catches. They are *Mugil cephalus, M. cunnesius, Liza macrolepis, L. parsia, L. fade, Ellochelon vaigiensis, Valamugil seheli* and *Rhinomugil covsula*. The other known species are *L. carinatus, V. buchanani, Sicamugil cascasia, Plicomugil labiosus* and *Crenimugil crenilabis*. Mullets are caught along the sea coast, in the lagoons and the adjoining brackish-water lakes, and in the estuaries. As they are caught almost throughout the year, they are a valuable source of food-fish during the offseason of the other commercial fisheries. Since mullets in general are hardy fish they are best suited for fish farming through which could be obtained better increments in growth and a ready source of fish. Mullets are usually distinguished by the presence of two separate dorsal fins, small triangular mouths, and the absence of a lateral line organ. They feed on detritus and most species have unusually muscular stomachs and a complex pharynx to help in digestion. Mullets are caught in cast nets, dip nets and seins almost throughout the year and contribute to the fishery in estuaries, backwaters and sea. (G. Luther, 1973)

However, this species appears to be rare in the Hooghly-Matlah estuarine system. In the Chilka Lake this is the most common mullet and fishing season extends almost throughout the year. It is mostly caught by Jano fishing. Fish with roe is common during September-January forming a peak in October-November. This species undertakes seaward breeding migration from September/October to December, when sizes between 35 and 53 cm are common in the catches. In the Mahanadi estuary, the species is available throughout the year, with the peak fishing season during September-November/December. In the Pulicat Lake, this is very common in the mullet catch, and is abundant during April, June-July, December and March with the dominant size between 20 and 43 cm. Fish with roe occurs for a few months from November onwards. At Mandapam sizes between 7 and 31 cm are commonly caught from the Palk Bay and the Gulf of Mannar. In the Kayamkulam and the Vembanad Lakes the species is very common, occurring in the size range of 23-61 cm. and with roe from October to January in the former. Fry of about 25 mm are abundant from November to February, fingerlings 40-70 mm being common during January-February in the Chilka Lake. In the Mahanadi estuary, post-larvae of 12-13 mm occur from January to April. In the Pulicat Lake and at Mandapam fingerlings of about 70 mm are common in January. (G. Luther, 1973)

Food and Feeding

Grey mullet is a diurnal feeder, consuming mainly zooplankton, dead plant matter, and detritus. Larval *M. cephalus* are planktonic feeders in the offshore marine environment and when they reach the size of about 20 mm SL, they undergo change in diet and started feeding on benthic organisms. Mullet have thick-walled gizzard-like segments in their stomach along with a long gastrointestinal tract with multiple pyloric caeca that enables them to feed on detritus. They are an ecologically important link in the energy flow within estuarine communities. Feeding by sucking up the top layer of sediments, flathead grey mullet remove detritus and microalgae. They also pick up some sediment which functions to grind food in the gizzard-like portion of the stomach. Mullet also graze on epiphytes and epifauna from seagrasses as well as ingest surface scum containing microalgae at the air-water interface. The amount of sand and detritus in the stomach contents increases with length, indicating that more food is ingested from the bottom substrate as the fish matures. (FAO, Fisheries and aquaculture)

Growth

It has been reported that the larvae hatch at approximately 2.6 mm in length and attain 17.7 mm by 42 days. Overall growth is rapid in the first year, with fish attaining 140-180 mm SL in tropical and sub-tropical waters (Thomson, 1963) and 130-160 mm SL in more temperate regions. The maximum size recorded for the grey mullet is 68-72 cm TL from sub-tropical waters of Lake St. Lucia (Wallace, 1975). Usually Grey mullet attains approximately 300g in the first year and 1.2 kg in the second year.

Migration and Life history traits

Grey mullet is catadromous, frequently found coastally in estuaries and freshwater environments. Mullets are generally schooling fish and the migration by the adult grey mullet occurs during the breeding season in different parts of the world. Along the West African coasts, adult grey mullet can take reproductive migrations of over 400 km (Bernardon and Wall, 2004). Often the shoals of the ripe mullet congregate in the mouth regions of the estuaries before moving out to the sea (Wallace, 1975). After spawning, the spent fishes came back to the estuaries. Usually the larvae are seen near the sea surface between the coast and the continental slope over the shelf (Ditty and Shaw, 1996).

Young grey mullet first enter the estuaries when the individuals are between 15 and 25 mm SL size and recruitment is only for short periods. The timing of *M. cephalus* recruitment in to the estuaries coincides with onset of favourable conditions within the nursery area usually after the rainy season which ensures productive marine larval habitats (Payne, 1976; De silva and silva, 1979). In most parts of the world, *M. cephalus* spawns in the near shore marine environment, the egg and early larval stages are spent drifting in ocean currents. Schools of these fry enter estuaries after a month at the sea (Hsu et al., 2009) and colonise the entire length of these systems. The juvenile and sub-adult life stages are spent mainly in estuarine waters and the adults then emigrate to the sea to spawn.

Reproductive biology aspects of *Mugil cephalus*

Oviparous teleost fishes can be separated into two groups according to their spawning strategy: the semelparous fishes, which have the a single spawning event during their lifetime such as some species of salmon (Crespi and Teo 2002) and the iteroparouus fishes, which have several breeding events during their lifetime. Iteroparous species can be divided into two sub categories (1) the annual spawner i.e., reproduce only once during the breeding season each year. (2) the annual multiple spawners i.e., reproduce several times during the breeding season each year. *M. cephalus* is annual single spawner and also considered as an isochronal spawner (Greeley et al., 1987; Render et al., 1995).i.e., all the developing oocytes in the ovary are at the same stage.

Gonadal development up until an advanced stage occurs in the estuarine waters but ripe running stage is generally only attained in the marine environment (Bok, 1979; Wallace, 1975). If mature fishes are denied access to the sea during the spawning season, grey mullet tend to resorb their gonads (Wallace, 1975). However, the success of fertilization and larval survival of grey mullet depends on the environmental salinity and maximum larval survival is reported in the salinity range of 30-40 ppt (Lee and Menu, 1981).

Size at first maturity and Fecundity

The size at maturity reported for the species ranges very widely from 22 cm SL from High brackish lagoon, Southwest Nigeria (Soyinka, 2014) to 43 cm TL from Black sea (Okumus and Bascinar et al, 1997).

The fecundity ranges from 0.2 million eggs to 3.89 million eggs from grey mullets of size 33 cm TL to 58 cm TL respectively (McDonough et al, 2003).

Maturity stages of ovary of *Mugil cephalus*

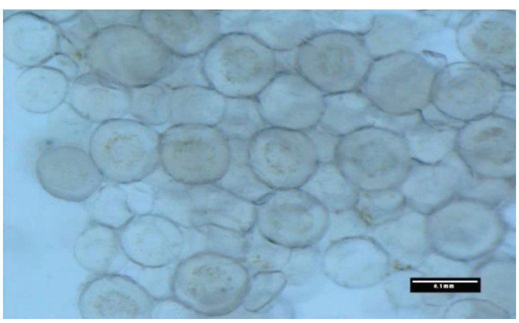
In *M. cephalus,* the external sex distinguishing characters are absent. Generally the males are slender and smaller than females. During the breeding season, oozing males and females with large, flabby abdomen (Gopalakrishnan, 1991) could be found. The ovaries are paired, elongated and covered by thin peritoneal membrane. The lobes are slightly asymmetrical and the left lobe is smaller than the right lobe. Posteriorly the ovaries are fused and open to the exterior through a common urogenital aperture just behind the anus. The both lobes are separable and attached to the dorsal coelomic wall by a thin membranous mesovarium.

The maturity stages can be classified based on shape, size and colour and a five point scale is used to describe the maturity stages. The maturity stages of the ovary can be divided into the following stages.

1. Immature

The ovaries appear as reddish, translucent structures united at the posterior end. The entire gonad occupies one fourth of the body cavity. The oocytes correspond to previtellogenic oocytes and are characterized by a small size.

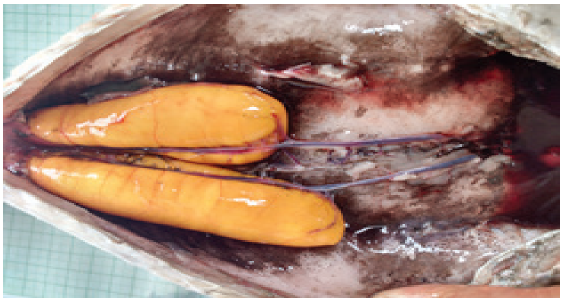
Immature ovary inside the body cavity



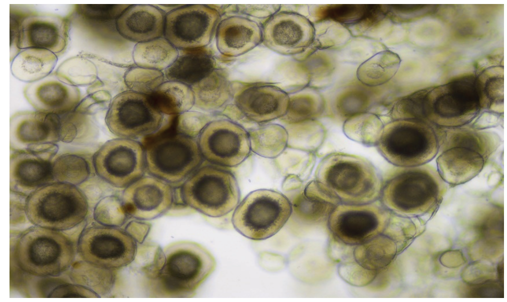
Immature ova are seen under light microscopy.

2. Maturing

Gonads fill almost half of the abdominal cavity and arteries are clearly visible. Ovaries are reddish yellow with a granular appearance. This stage indicates the onset of vitellogenesis (endogenous and exogenous) and can be easily distinguished from stage 1 oocytes by the presence of lipid droplets in the peripheral ooplasm (endogenous vitellogenesis). The average diameter increases and this stage are generally identified at its beginning by the appearance of small yellow spots visible to the naked eye that correspond to the stage 2 developing oocytes. At the end of the stage 2, the ovaries are completely filled up with clearly visible yellowish oocytes.



Maturing ovary inside the body cavity

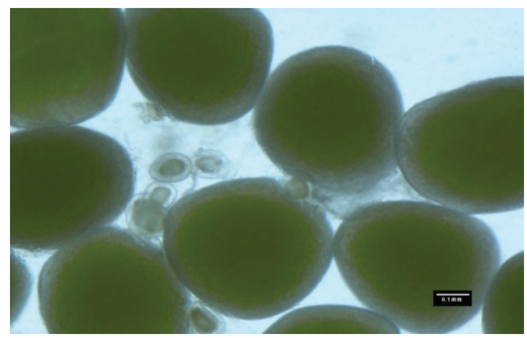


Maturing ova are seen under light microscopy.

3. Advanced maturation / Mature

A pair of distinctly orange yellowish cylindrical ovarian lobes is visible. The entire gonad occupies three fourths of the body cavity and is fully packed with yolky oocytes. The ovarian wall appears as very thin, distended and almost transparent. Ova are yellowish, granular, round, yolk laden and appear as dark bodies under microscope. These ovaries characterize fully vitellogenic females, close to the spawning period.

Matured ovary inside the body cavity

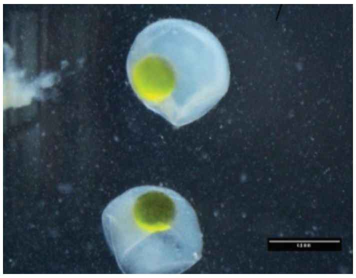


Matured ova are seen under light microscopy. Arrow indicates immature ova

4. Ripe

Ripe oocytes occur immediately before ovulation. The duration of this stage is short as the female is undergoing the final rapid development of oocytes before ovulation. The oocytes are characterized by the migration of the nucleus to the animal pole, and fusion of the yolk globules and oil droplets. Finally, the yolk appears as a homogenous mass filling the interior of the oocytes. Before spawning, the oocyte in the ovary are not transparent, but simultaneously on gaining transparency, the eggs sharply increase in volume and their specific density increases, allowing them to float in seawater of normal density i.e., an egg become pelagic (Fulton, 1891). In fishes spawning in sea water, the egg volume increases by several folds, sometimes by hundreds of times (Wallace and Selman, 1981). The increase in water content i.e., hydration serves as primary cause for volume and weight gain in a follicle. It is believed that hydration of oocytes in teleost fishes during maturation is a unique phenomenon among vertebrates (Wallace an Selman, 1978).

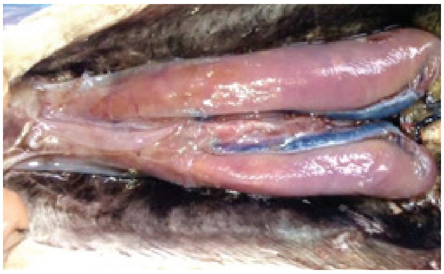
Ripe ovary inside the body cavity



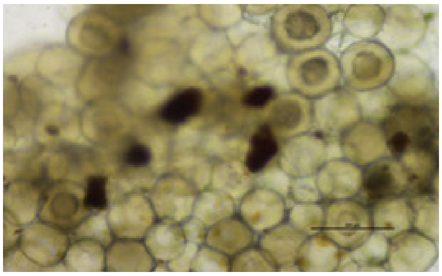
Ripe ova are seen under light microscopy.

5. Spent

The ovaries are purple in colour, highly shrunken, collapsed and occupy approximately half of the body cavity. They appear flaccid and loosely packed with primary oocytes. Ovarian wall is thick. The immature stock of ova can be seen along with few large disintegrated yolky oocytes which undergo the process of resorption. Blood shots are common in spent ovaries.



Spent ovary inside the body cavity



Ova in the spent ovary are seen under light microscopy.

Maturity stages of male *Mugil cephalus*

The testes are attached to the peritoneal cavity by means of mesorchium and protected peripherally by thin connective tissue, the tunica albuginea. These are tubular, restricted type and consisted of vasa differentia and a common sperm duct. The two lobes of the testis are more or less of same length. The stages are divided in to five stages based on colour, shape and size and classification is adapted from Elizabeth, 1987 with some modifications.

1. Immature

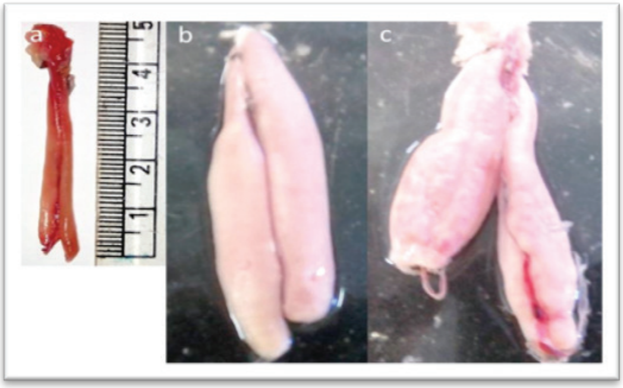
The testis appears as two threads like structures, united at the posterior end. They are semi-transparent and measure about 5 to 7 cm in length. They occupy almost half of the body cavity.

2. Maturing

The testes becomes more flattened, ribbon like, appears opaque with smooth surface. The entire gonad occupies about two thirds of the body cavity.

3. Mature

This stage is characterised by the white, turgid and opaque testes with smooth surface. Size of the testis is almost similar to that of the previous stage. Small amount of milt oozes out when pressure is applied to the abdomen. Difference in lobe length is common in this stage.



M. cephalus testes at different maturity stages a) Immature b) Maturing and c) Mature

4. Ripe/Oozing

The size, shape and colour are similar to the previous stage. But the testes appear more turgid and the milt oozes out freely with slight application of pressure to the abdomen

5. Spent

The testes appear flaccid with surface thrown in to folds. Only a little quantity of the milt oozes out on application of considerable amount of pressure to the abdomen.

Conclusion

M. cephalus is a detritivore feeder with well-developed digestive system and occupy the lower trophic level in the food chain contributing significantly to the energy flow in the estuarine systems. The capacity of fish to survive in aquatic habitats of varying salinity, turbidity and dissolved oxygen levels makes it suitable candidate for freshwater, brackishwater and marine farming. It is a high fecund fish with group synchronous ovary; usually spawns once in the breeding season. The normal development of gonads in the captivity is influenced by many factors like salinity, temperature, day length, nutrition, handling stress and hormone dose; sometimes the development of the gonads is stopped due to the unfavourable conditions, thus leading to atresia (resorption of the oocytes and spermatocytes).

PROCUREMENT, TRANSPORTATION AND ACCLIMATIZATION OF FISHES FOR BROODSTOCK DEVELOPMENT

Aritra Bera, Krishna Sukumaran , M.Makesh , Tanveer Hussain, Dani Thomas, M.Kailasam

Introduction

Successful seed production in the hatchery depends upon the availability of healthy matured fishes. For selecting potential breeders, viable broodstock under captive conditions has to be developed. Since seabass attains maturity after 2 years of age to develop broodstock from farm grown fish, one has to wait more than 2 years. To save time, adult fishes could be procured from the commercial catches, transported carefully to the hatchery holding facilities and maintained. Whether from the farm or from the wild catches, fishes have to be procured with care, transported and follow some protocols for healthy broodstock development.

Procurement

Adult Sbrood fishes can be procured from wild catch or from the farm harvest. The selection of a suitable gear or method of capture is very important in order to avoid injury to the fish. Mullet is usually caught using drag net, dip net, seine net andcast net Dip net is the best gear for brood stock fish collection because, dip nets cause less injury to the fish. But operation of this gear is not possible in many places.

Criteria for selection of fishes for broodstock development

1. The fish should be devoid of external injuries or internal haemorrhage.
2. Fins should be complete and there should not be any loss of scales
3. The jaw, snout, opercular region, eyes and gills should have not damaged, since these parts are vulnerable to injuries during capture by different gears and would lead further infections after released into the holding tanks.
4. The fish should be healthy and free from any parasite infection.

Transportation

Fishes procured have to be carefully transported. If the procurement site is nearby, transportation can be done manually. Fishes from distant places have to be transported using transporting vehicles. Transporting vehicles with water holding facility and inlet and outlet provision are to be selected. Inner lining with soft materials like foam is preferable so that the fish will not get injured while moving. There should be provision for oxygen cylinder for providing oxygen while on transportation. While transportation, anesthetic agents like Aqueous or Chilaldine or MS 222 or Phenoxy ethanol may be used at lower concentration to keep the fishes on rest.

Manual transportation from nearby areas is done by simply transporting the fishes using buckets or troughs with water covering with perforated lids in the case of small fishes. If the fishes are large, (more than 2.0 kgs in size), they can be transported using specially designed transport tubes. Large rubber tubes specially designed with perforation for water exchange can be used. Fishes can be allowed into the tube and can be manually toed along the water-line to reach the hatchery. This would avoid injury to the fish.

Acclimatization

After the fishes are brought to hatchery/ holding facility, they should be released in the acclimatizing tanks with quality filtered seawater of the same salinity, temperature etc., of the transported medium and mild aeration should be given. Since the fish would be under stress due to transportation, they can be kept in this tank for 1 – 2 hrs with flow through. After normalization, the fish can be treated with acriflavin (1 ppm) for 10 minutes and or with (5 ppm) $KMNO_4$ for one hr as prophylactic treatment to avoid infection on minor injuries, in any. The fish can be kept under hatchery condition 3-5 days for close observation and later on can be shifted to brood stock holding facility for further maintenance.

Quarantine

Fishes collected may have some parasites or other pathogenic infections. To make sure they are devoid of pathogens fishes have to be kept under quarantine condition in separate tanks. If any fish develop symptoms of any disease, they should not be used for broodstock.

Conditioning of broodstock

The fishes brought for broodstock development would have lived in different conditions feeding with various types of feed. In the confined broodstock holding tanks or cages or ponds, they may not be getting the feed they were used to feed. So, the fishes have to be slowly conditioned to feed upon the feed which will be provided in the hatchery conditions by slow weaning, which may take few days for accepting the new feed.

Broodstock holding facilities

Healthy broodstock fishes after observing as protocols can be transferred to broodstock holding facilities like RCC tanks (preferably large tanks of 50 – 100 tonne capacity) or cages or ponds for further maintenance and development providing required feed, quality water and nutrition feed for maturation and spawning.

CAPTIVE BROODSTOCK MAINTENANCE OF MILKFISH (*CHANOS CHANOS*)

Tanveer Hussain, Aritra Bera, Babita Mandal, T. Sivaramakrishnan, M.Kailasam

Introduction

Milkfish, *Chanos chanos* is an important food fish traditionally cultured in Southeast Asian countries, particularly the Philippines, Taiwan, and Indonesia. It is hardy, fast-growing, and euryhaline. Milkfish has the ability to grow in brackishwater, seawater and freshwater ponds and lakes. This fish consumes low protein pellet feed and grows up to 500 g size in 6 months. This is a boon for small and marginal farmers who can grow the fish at a low cost. Out of 400000 ha of milkfish farm that has been reported to exist in the world, 183000 ha are in Indonesia, 176000 ha are in Philippines and 15600 ha are in Taiwan. Brackishwater ponds and freshwater pens in south east Asia produce 3,46,000 metric tonnes of milkfish annually. During recent years, Small scale attempts have been made for culture of this species using wild fry on small scale in the coastal regions of India, particularly, in traditional farming systems such as Pokkali fields, kharlands and bheris. The fry collected from natural water bodies could not meet the demand of farmers and also it carries unwanted weed and predatory fish. The demand for milkfish fry has spurred studies on broodstock development, artificial propagation, and mass fry production in hatcheries to supplement the natural supply and eventually break the dependence on wild sources. The difficulty in obtaining sexually gravid milkfish from the wild and the limited success in induced maturation and spawning prompted the development and establishment of milkfish broodstock. Significant achievements in the development of milkfish broodstock began in 1980 with the spontaneous maturation of 5 year old milkfish held in floating net cages (Lacanilao and Marte, 1980). Since then, milkfish has consistently spawned naturally in floating net cages (Marte and Lacanilao, 1986). Recently, 8 and 9-year-old milkfish have spontaneously matured and spawned in concrete tanks (Emata and Marte, 1990; 1991). Natural spawnings of milkfish in both holding structures, together with mass fry production using hatchery technology developed by SEAFDEC/AQD (Gapasin and Marte, 1990), could definitely supplement and ultimately break the dependence on the natural supply of milkfish fry. In India, ICAR-CIBA has achieved a breakthrough in artificially breeding of milkfish in captivity for the first time in the country during 2015.

Reproductive biology

Captive milkfish mature and spawn at 5 years of age. Tank or cage-reared adult milkfish weigh around 2.5-9.0 kg. Among captive females, the ratio of gonad weight relative to body weight (gonadosomatic index, GSI) ranges from 0.05% (immature) to 4.46% (mature) (Marte, 1989). In contrast, mature ovary can take as much as 25% of body weight of wild-caught milkfish (Bagarinao, 1991). Captive females produce an average of 200,000 eggs/kg (Marte and Lacanilao, 1986). Immature captive males have a GSI of 0.1% that increases to 4.0% as maturity is attained. In captivity, milkfish broodstock are immature in December and January, gonadal development begins in February and March, and spawning occurs from April to November. This pattern is consistent with the occurrence of milkfish adults, eggs, and fry in coastal areas (Bagarinao, 1991).

The spawning season appears to coincide with a long photoperiod and relatively high temperatures (Marte and Lacanilao, 1986).

Sources of broodstock and Holding facilities

Milkfish juveniles (weight, 250-350 g) can be obtained from brackishwater ponds and fish pens. Milkfish juveniles are transported to the broodstock cages or tanks in a floating fish cage or in a 1.5 m dia. Land-based concrete tanks measuring 12 m x 6 m x 2 m deep are sufficient for milkfish broodstock. A double pipe drainage is installed to allow water to flow out from the bottom. Water inlets and aeration lines are located at the top. Daily water inflow should be adjusted to change at least 50% of the water volume. The sides and bottom of the tank should be brushed monthly. The tank can be drained to at least a foot in depth for brushing. During the spawning season, water inflow should be increased so that brushing and draining can be minimized. In both tanks and cages, optimum stocking density should not be more than 1 kg/m^3.

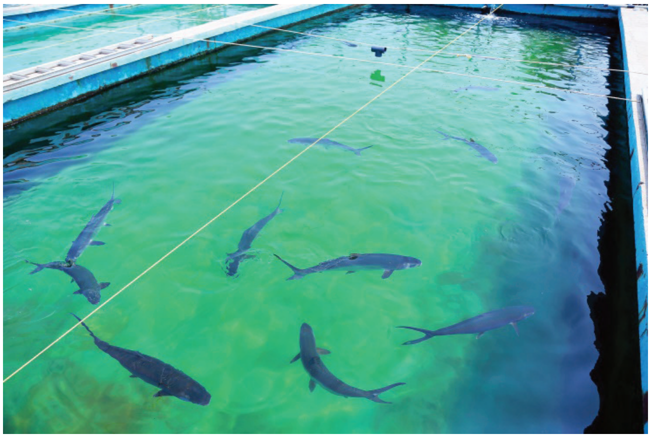


Fig : Milkfish Broodstock Tank (144t capacity)

Brood Nutrition

Two to four year old milkfish can be fed twice daily with commercial fish pellets (24% protein) at 3% of their total body weight. Upon nearing maturation by the fourth year, fish are fed twice daily with pellet feed with 36-42 % protein and 6-8 % lipid. Daily feeding ration is increased to 4% of total body weight.

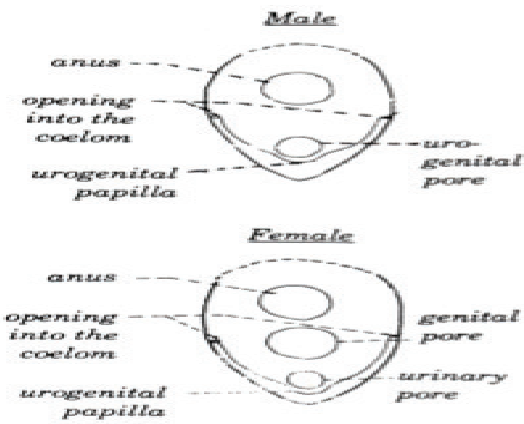
Determination of gonadal development

From the fifth year and thereafter, the fish are sampled in March and in November to determine the stage of gonadal development and to determine the sexual composition of the stock. A sex ratio of one female to one male or two females to one male is adequate for egg production. Unsexed fish can be kept in a separate cage or tank to be sexed later in the season or in the next season.

The stage of gonadal development can be monitored through the cannulation biopsy technique. The nets of the floating cages are lifted gradually, or the concrete tanks drained. Disturbance must be minimized to prevent any physical injury to fish. Fish are individually scooped out and then placed in a 400 L FRP tank containing 200 l seawater and 200 ppm (40 ml) 2-phenoxyethanol (an anesthetic). The anesthetic should be mixed with a little water before it is placed in the tank. Anesthetized fish are characterized by loss of balance (ventral side up), immobility, and rapid and shallow opercular movement.

Sexual Dimorphism

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



Source: - Anal region of Milkfish (Chaudhuri *et al.,* 1977)

Cannulation biopsy

In the wooden trough, the fish is laid on its dorsal side. To determine the presence of white viscous milt, its ventral side is gently pressed, starting from halfway of the abdomen to the anal region. If no milt oozes out, a cannula (polyethylene tubing, PE 100; inner dia., 0.86 mm; outer dia., 1.52 mm; Clay Adams, New Jersey, USA) is inserted into the genital pore, the second opening in the urogenital area relative to the head. The free end of the cannula is held in the mouth. The cannula is aspirated while slowly being withdrawn from the fish. The cannula is immediately inspected. A milky whitish substance indicates a maturing or mature male. Spherical yolky oocytes appear translucent to opaque (quite distinct from fatty tissue which lines the abdominal wall). Cannulated gonadal tissue is then blown into a small covered tube (e.g., microcentrifuge tube) and 5% formalin solution is added to preserve oocytes for examination and measurement. A few oocytes are pipetted from the microcentrifuge tubes and placed on a glass slide. The diameter of 10-30 oocytes are measured under a microscope provided with an ocular ruler. Oocyte size indicates degree of sexual maturity. Females with an oocyte diameter equal to or greater than 0.67 mm are considered near final maturation and spawning.

Conclusion

Milkfish being herbivorous euryhaline species is emerging as prime candidate species species which is highly suitable for farming in brackishwater as well freshwater ponds. This fish can be farmed by using low protein pellet feed and grows up to 500 g size in 6 months. This is a boon for small and marginal farmers who can grow the fish at a low cost. Milkfish fetches Rs. 120-150/kg in the local market while the production cost is ranged from only Rs. 60-70/kg. Proper broodstock management strategies with provision of high quality broodstock diet periodic assessment of maturation and health status successful seed production of milkfish can be achieved on large scale basis.

BROODSTOCK DEVELOPMENT AND INDUCED CAPTIVE MATURATION OF GREY MULLET *MUGIL CEPHALUS*

Krishna Sukumaran, Rekha MU, Dani Thomas, Aritra Bera, T. Sivaramakrishnan,
M. Makesh, M. Kailasam

INTRODUCTION

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

BROODSTOCK MAINTAINENCE

Broodstock holding system

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

Broodstock availability

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

Procurement of brooder

Brooders of grey mullet can be procured from the wild or raised in ponds from early stages however the latter involves an investment of tremendous effort and time. A sizeable broodstock of grey mullets are desirable. Grey mullet seem to exhibit a state of social hierarchy in which only a small fraction of dominant females mature (less than 20%) supressing the maturity of the con-specifics. This makes only a small number of mature females available for use during the induced breeding in the season. This implies that that a sizeable broodstock should be maintained in the holding systems.

Broodstock selection

Males of grey mullets mature between 250- 300 mm standard length while females mature at slightly larger size, 270- 350 mm. Males are reported to mature at approximately 2-3 years of size while females mature at 3-4 years. A minimum fork length of 310 mm or three years of age is suggested best for selection of broodstock.

Environment and water quality

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

Natural feed and formulated feed

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

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

Prophylactic treatment

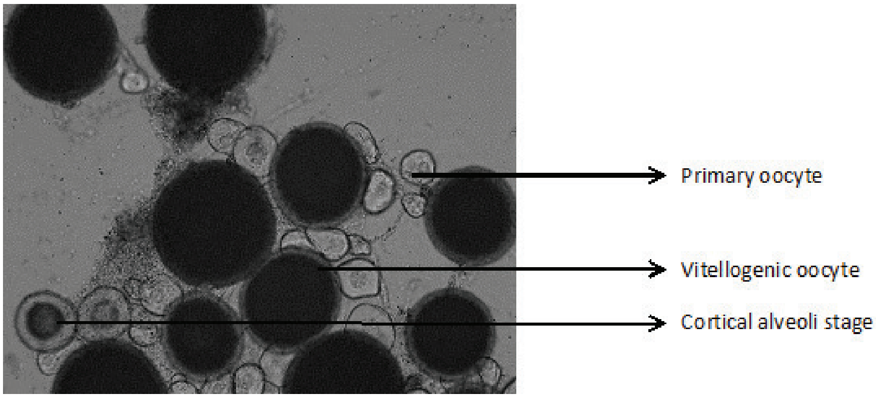
Grey mullets can act as hosts for a number of parasites. Visual observation and periodic examination of the fish for parasites is conducted. Fish should be carefully observed daily for reduction in feed intake and swimming activity as signs of parasitic infection. Periodic chemical treatment on a monthly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

REPRODUCTIVE BIOLOGY

Grey mullets spawn only once every annual reproductive cycle and are termed single batch group synchronous species. They have a relatively confined reproductive season. Spawning grounds of grey mullets are located in the sea. Grey mullets are generally reported to spawn once a year and exhibit synchronous ovarian maturation. Fecundity is reported in the range if 1.2- 2.8 million for the species (Thompson 1983) and 0.5-2 million (Bester 2009) and 849 eggs per g body weight (Nash et al., 1974). The optimum temperature reported for egg development of grey mullet is 24 ^{0}C. However, with optimal hormonal treatments a maturity of single female was manipulated to make in spawn twice in a season at Oceanic Institute Hawaii.

MATURITY ASSESSMENT

Maturity assessment of the broodfish is conducted with the approach of the spawning season. The fish after being caught carefully and anaesthetised in 30 ppm 2-phenoxyethanol. These fish are cannulated to assess the ova diameter and the condition of the eggs. In case of males, gentle pressure is applied on the abdomen to check for milting two to three times. Males which express milting during the very first press is selected for the trials. The stage of maturity in female fish is assessed by biosying the oocytes. This helps in judging the right stage of maturity and giving the appropriate hormonal treatment. Captive grey mullets found to possess an oocyte size about 80-90 µm were found to be in the primary oocyte stage, 110-120 µm in the slightly advanced stage of the primary oocyte, the perinuclear stage. Oocytes in the size range of 140- 150 µm were found to be in the cortical alveoli stage. Further, oocytes above 180 µm were seen to be vitellogenic. This stage is good for administering LHRHa implants for supporting oocyte development to functional maturity. In grey mullets, an ova diameter of 600 micro-m is reported to be optimum for successful induced spawning.



INDUCTION OF CAPTIVE MATURITY

Many fish species exhibit reproductive dysfunction under captive conditions. These may either be i) the failure to undergo normal vitellogenesis ii) the failure to undergo final oocyte maturation. The former is a more serious challenge and observed in species like the European eels.. In case of grey mullets it is the failure to undergo final oocyte maturation which is the commonly encountered reproductive dysfunction. The dysfunctions maybe attributed to unavoidable stress in captivity, lack of natural environmental stimuli etc. To some extent these may be controlled by simulating the natural environmental factors such as photoperiod or temperature or maybe salinity. We know that in grey mullets a photoperiod of 6d/18L and a temperature of 21^{o} C was found to stimulate vitellogenesis and a fish of functional maturity can be attained. However, it is often impractical and expensive to simulate all the required parameters like water depth, current patterns or natural migration of the fish. Hence, in captivity we encounter the challenge of the female fish failing to undergo final oocyte maturation and the males exhibiting diminished production of quality milt. This is also true for grey mullet broodstock held in captivity.

We currently rely on use of sustained releasing exogenous hormone implants for optimising the percentage of mature fish in captivity and minimising the occurance of follicular atresia. This is done through the use of sustained release of LHRHa in case of female fish which is in the early stage of vitellogenesis. LHRHa acts on the pituitary gland to stimulate the release of gonadotropic hormones (GtHs) which in turn stimulates the gonads to produce steroid hormones for gamete production. There are two types on gonotropic hormones; GTH-I and GTH-II. The former, the follicle stimulating hormone supports the initial phase of gametogenesis. In case of female fish vitellogenesis is promoted. Vitellogenesis involves the sequestration of yolk precursors in oocytes. The process is also accompanied by oocyte growth attributed to the uptake of these yolk precursor proteins, the vitellogenin and also the low density lipoproteins. In case of males fish, the steroid hormone; 17-ἀ- methyl-testosterone is utilised to directly act on the gonads to promote androgen production and maturation of the testes. Commonly a GNRHa based delivery system may be used about 6 weeks before the peak spawning season.

Methods used for sustained hormone delivery in grey mullets

A variety of GNRHa delivery systems are developed, one of the first such systems are cholesterol based pellets. In Israel, for enhancing gonadal maturity ethylene-vinyl acetate copolymer (EVAc) slow release implants were used to promote maturity of grey mullets in captivity. At MES, CIBA we use cholesterol pellets and Silastic implants for sustained hormone delivery to promote gonadal maturity of grey mullets.

Cholesterol pellets- This is used for sustained delivery of the neuropeptide LHRHa in female fish. The exposed surface area of the pellets and the relative composition of the pellets influence the release rate of the hormones. Cholesterol based pellets of LHRHa, 200 micro-g is suggested for implantation in the dorsal musculature of the fish (Tamaru et al., 1989). Use of a combination of LHRHa and testosterone pellets have been shown to result in accelerated oocyte growth.

Silastic implants- This is used for sustained delivery of steroid hormone, 17-ἀ- methyl-testosterone in male fish. The hormone may be used after dissolving in castor oil or in crystalline form in the silastic implants.

For males silastic implants containing 17-α-methyl-testosterone containing 10 mg 17- α-methyl-testosterone has been found effective for 10 months in inducing testicular maturity (Lee, 1992).

Captive maturity percentage of grey mullets brooders maintained in broodstock tank system at MES, CIBA have reported a maturity percentage over 50% with the support of slow releasing hormone implants. An oocyte growth rate of approximately 7 micro-m per day is recorded after hormonal implantation of grey mullet females in the tank systems of MES, CIBA. Silastic implants have supported in sustaining male maturity of mullets throughout the breeding season, however not beyond the season.

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DEVELOPMENTOFFORMULATEDBROODSTOCKFEEDFORMILKFISH (*CHANOS CHANOS*) AND GREY MULLET (*MUGHIL CEPHALUS*)

K. Ambasankar, T. Sivaramakrishnan, K. P. Kumaraguru Vasagam, J. Syama Dayal, and K. P. Sandeep

Introduction

The rapid industrialization of milkfish and grey mullet culture was accompaniedby gradual shift in production systems from extensiveand semi-intensive systems to more intensive systems, withgrowing demand for formulated feeds.This expansion has also created a gap between quality seedsupplies and farmers' demand. Therefore, the production ofsufficient quantities of quality seed and the formulation ofappropriate broodstock and larval feeds have become amajor challenge facing the brackish water finfish culture industry.

Milkfishand mullet broodstock nutrition has attracted the attention asone of the major factors limiting seed production and larvalquality. The spawning performanceand seed production of milkfish are directly related to broodstocknutrition. The essential nutrients required for gonadal developmentof finfish broodstock and for the performance of theproduced seed are provided by exogenous feeding. Therefore, inadequate,or poor quality broodstock feed, will likely lead topoor spawning performance, seed production and larval performance.

For quicker maturation, the broodstock fishes are to be fed with highly nutritive diet. Diet rich in vitamins, poly-unsaturated fatty acids (n- 3 PUFA) and other micro-nutrients is essential for obtaining viable eggs and larvae. During gametogenesis, female fish require a food, richer than usual, in proteins and lipids to produce the vitellogenin. As the sole source of food for the developing embryo and the early larval stage until feeding on live preys starts, yolk quality and quantity are key factors for a successful reproduction. Both dry pellets and moist food are also employed during maturation. Dry pellets should include essential nutritional components like polyunsaturated fatty acids (n-3 PUFA), in particular EPA (20:5 ω 3) and DHA (20:6 ω 3), which cannot be produced by fish metabolism.

All animals including fish require food to supply the energy that they need for movement and all the other activities that they engage in for growth. However, they are 'cold-blooded' and as their body temperature is the same as the water they live in, they do not therefore need to consume energy to maintain a steady body temperature and they tend to be more efficient users of food than other farm animals. The nutrient requirement of different species of finfish vary in quantity and quality according to the nature of the animal, its feeding habits, size, its environment and reproductive state.This is the reason for understanding the nutritional requirement of candidate species which assumes paramount importance in developing the feeds for the candidate species.

Nutritional requirement of brackishwater finfish species

Protein

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

Table 1. Nutrient requirements of milkfish and Grey mullet

Nutrient	*Chanoschanos*	*Mugilcephalus*
Energy (Kcal/kg)	3500-4000	4000-4500
Protein %	30-40	27-35
Lipid %	10	6-9
Carbohydrate %	25	30-40

Amino acids

The growth of fish is directly related to the quality of protein in terms of amino acids. After digestion of protein, amino acids are metabolized at tissue level to form new proteins for growth, maintenance and energy. Among 25 amino acids present in protein 10 amino acids must be supplied in the diet since fish cannot synthesize them and termed as essential amino acids (EAA). These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. A large proportion of the amino acid consumed by a fish are catabolized for energy and fish are well adapted to using an excess energy in this way. It is found that if the amino acid composition of the protein in the feed matches with the amino acid composition of shrimp body tissue, such feed promotes good growth.

Lipid

Lipid is a complex mixture of simple fat, phospholipids, steroids, fatty acids and other fat soluble substances such as pigments, vitamins A, D, E and K. Apart from its major role to supply energy lipid also act as precursors to many reactive substances. Phospholipids are responsible for the structure of cell membranes (lipid bi-layer). Fatty acids are the main active components of dietary lipids. Deficiency of essential fatty acid result in general reduction of growth and a number of deficiency signs including depigmentation, fin erosion, cardiac myopathy, fatty infiltration of liver and 'shock syndrome' (loss of consciousness for a few seconds following an acute stress. Fat levels of 6-8% are adequate in most of the fish diets. However, the quality of fat in terms of fatty acids is more important. However, lipid level should be adjusted in diet considering the technological problems in feed manufacture and storage. Fish oil and soya oil are generally used as lipid source during feed formulation.

Fatty acids

Fish are unable to synthesize fatty acids of the n-3 and n-6 series and must be provided in their diets. Aquatic animals require higher n-3 fatty acids than terrestrial animals. Among aquatic animals, marine habitat requires more HUFA than freshwater counterparts. Among the long chain fatty acids polyunsaturated fatty acids (PUFA) such as linoleic acid (18:2n6), linolenic acid (18:3n3), eicosapentaenoic acid (20:5n3) (EPA) and docosahexaenoic acid (22:6n3) (DHA) are essential for growth, survival and good feed conversion ratio. The n3 fatty acids are more essential than the n6 acids. The fatty acids, EPA and DHA, which are known as highly unsaturated fatty acids (HUFA) of n3 series, are particularly important. Quantitatively EPA and DHA are needed at 0.5% and 1.0% in the diet of larvae and fry of brackishwater fish. Fresh water fish show requirement for n6 and n3 essential fatty acids (EFA), whereas marine fish show requirement of n3 and also HUFA.

Phospholipids

Fish require phospholipids for growth, metamorphosis and maturation. Lipids of squid, clam, shrimp, fish and polychaetes are excellent natural source of phospholipids. The phospholipid phosphatidylcholine (lecithin) is essentially required in the diet of larval and fry stages of fish for fast growth and good survival. Soya lecithin is a good source of phospholipid. It is required at 1-2% level in the diet. The development and survival of larvae is significantly improved when the diet contains lecithin.

Carbohydrate

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

Vitamin and Mineral requirement

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

Fish can absorb minerals directly from aquatic environment through gills and body surfaces or by drinking. Hence, dietary requirement of minerals is largely dependent on the mineral concentration of the aquatic environment. About 20 inorganic elements (macro and micro) are required to meet the metabolic and structural functions in the body of animals. The aquatic organisms regulate the mineral needs through dietary source and also through internal regulatory mechanisms in the kidneys and gills. Trace minerals like copper (Cu), cobalt (Co), selenium (Se), iodine (I) and chromium (Cr) have some role in general upkeep of the organism. Their dietary incorporation enhances growth and survival.

Table 2. Vitamin requirements of fish

Vitamin (mg/kg)	Fish
Thiamin	10
Riboflavin	20
Pyridoxine	10
Pantothenic acid	40
Niacin	150
Folic acid	5
Vit B_{12}	0.1
Choline	3000
Inositol	400
Vit C	100
Vit E	30
Vit A (IU)	2500
Vit D (IU)	2400
Vit K	10

Milkfish Broodstock nutrition

Significant achievements on nutritional studies have been obtained to warrant initial formulation of a practical diet for milkfish which has been a major concern of the nutritionists. Milkfish fingerlings require a diet of 30-40% protein, 10% fat, and 25% carbohydrates. Energy levels exceeding 3,500 kilo calories per kilogram do not improve weight gain. Amino acid-supplemented diets containing protein-energy ratio to total metabolizable energy ratio of 44% promote highest growth rates of milkfish juveniles compared with any of the other ratios.

Based on dry diet, the optimum growth requirements of milkfish juveniles for essential amino acids are: lysine, 2.0%: arginine, 2.1 %; threonine, 1.8%; histidine, 0.8%; isoleucine, 1.8%; leucine, 2.3%; valine, 1.6%; and phenylalanine, 1.9% at tyrosine level of 0.45% and 1.3% at tyrosine level of 1.20%. The growth potential of a particular dietary protein source can be determined from the closeness of its amino acid profile to the growth requirements.

Milkfish has a total lipid content of 4.5% to 4.9%. Lipids consist mainly of neutral type, primarily triglycerides and cholesterol ester. Essential fatty acid requirements of milkfish indicate that (n-3) fatty acids such as 18:3(n-3) and (n-3) highly unsaturated fatty acids are nutritionally more important than 18:2(n-6). Milkfish fingerlings fed diets containing 10% vegetable protein had significantly higher growth rates, protein efficiency ratio, and survival rates than those given animal protein-containing diets.

Institute has developed a unique formulation for Milkfish Broodstock using novel and speciality ingredients and the feed has been found to be effective for milkfish breeding. The feed developed is also being used for Milkfish Broodstock byAditya Fish Hatcheries (Kakinada, Andhra Pradesh) under the collaborative research cum technology transfer program.

Mullet Broood[Plus]

Milkfish Broood[plus]

Grey Mullet Broodstock feed

Grey Mullet Broodstock feed a new broodstock feed for Flat Head Grey Mullet (*Mugil cephalus*) is available for commercialisation from CIBA, Chennai. This feed is a result of project work aimed at optimal broodstock feed for Grey mullet captive maturation in cement tanks. Special emphasis was an ovarian development in females and spermiation in males.

Focus in this broodstock feed development has been on achieving good number of the stock with vitellogenic oocytes three months before the breeding season and having sustained oocyte growth till spawning. The ingredients have been chosen to provide complete maturation enhancing factors.

Proteins, energy, lipids and fatty acids, minerals and vitamins have been incorporated according to the specific need of the maturing mullet during vitellogenesis. Various special additives have been included to provide natural maturation enhancers, pigments and gustatory stimulants. The formulation ensures complete utilisation of all nutrients by the fish for maintaining all round health and rapid gonadal growth. Selected high quality raw ingredients such high quality fish meal, fish oils, phospholipids, combination of high quality vegetable proteins provide balanced amino acids, Fatty acids (EPA, DHA), minerals and vitamins.

Having thoroughly studied the general reproductive physiology of this species, two types of formulations are provided-"feed for spawning season" and "feed for pre-spawning season". Using these feeds, large depositions of adipose tissue (storage fat inside the body) are encouraged which gets mobilised into oocytes as yolk during the process of vitellogenesis, ensuring large sized eggs and hatchings with sufficient endogenous nutrients for larval development, and thereby ensuring that the next generation of fish gets the best start in life.

This is a sinking feed (3 mm or 6 mm), since mullets like to feed on the bottom. At the same time, the high physical quality of the pellets manufactured using latest and sophisticated machineries contribute in maintaining the tank water clean and healthy environment. The feed formulae can be suitably adjusted if required to prepare floating feed, without compromising the quality. Floating feed with similar nutritional qualities can also be prepared with slight feed manufacturing procedures.

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CULTURE OF LIVE FOOD ORGANISMS: MICROALGAE, ROTIFERS, ARTEMIA AND COPEPODS

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Introduction

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

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

❖ **<u>Microalgae as live feed</u>**

Marine microalgae are the floating microscopic unicellular organisms which are generally free living, pelagic in the size range of 2 to 20µ. They play pivotal role in aquaculture as a means of enriching zooplankton for feeding fish and other larvae. In addition to protein and energy supply, they provide other key nutrients such as vitamins, essential polyunsaturated fatty acids (PUFA), pigments and sterols, which are transferred through the food chain. Therefore, microalgae are inevitable source of nutrition for fish larvae. Microalgae varied in their proportions of protein (6.6-52%), carbohydrate (5.5-23%) and lipid (7-23%).

➢ **Culture techniques**

Microalgae can be produced using a wide variety of methods, ranging from closely-controlled laboratory methods to less predictable methods in outdoor tanks. Various chemical media are used for indoor and outdoor cultivation. (Guillard's F/2 medium, Walne's medium, Johnson's mediumetc). There are five different stages in the growth of every microalga (Fig 1). The best quality microalgae can be harvested/utilized during the late exponential growth phase.

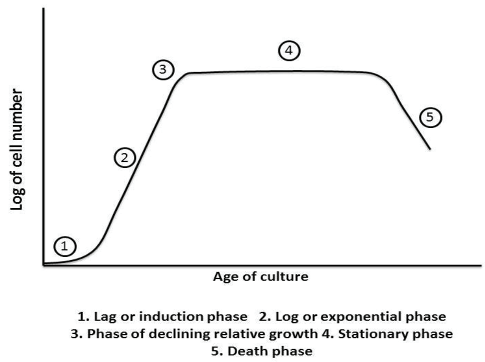

Fig: 1. Different phases in life cycle of microalgae

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

✓ Batch culture

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

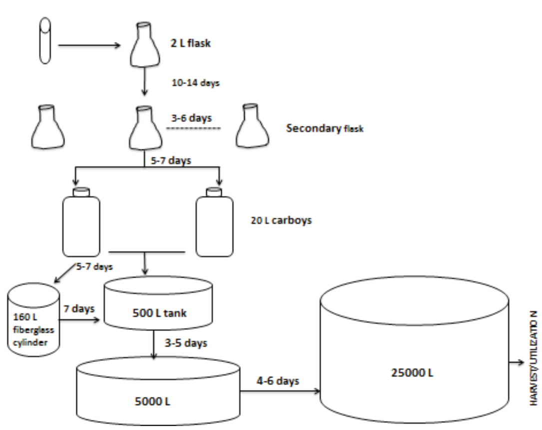

Fig: 2. Diagrammatic representation of batch culture of microalgae
(Source: FAO fisheries technical paper: 361)

✓ **Continuous culture**

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

✓ **Semi-continuous culture**

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

✓ **Microalgae production chain in finfish hatchery**

A finfish hatchery must be equipped with a well maintained indoor microalgae laboratory to keep pure cultures different strains used. The temperature, light intensity and aeration/CO2 supply should be controlled inside the lab. Sterilized media are used in the lab. The culture volume ranges from 10 ml to 20 litres in the lab. The inoculum for intermediate culture is provided from the indoor laboratory. Generally, 20 litre carboy cultures are used as inoculum for intermediate culture. The intermediate culture contains 500-1000 litre FRP tanks in a room with transparent roof and adequate ventilation. The cultures from intermediate room are used in large outdoor tanks (5-20 tons).

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

✓ **Nutritional perspective of microalgae**

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

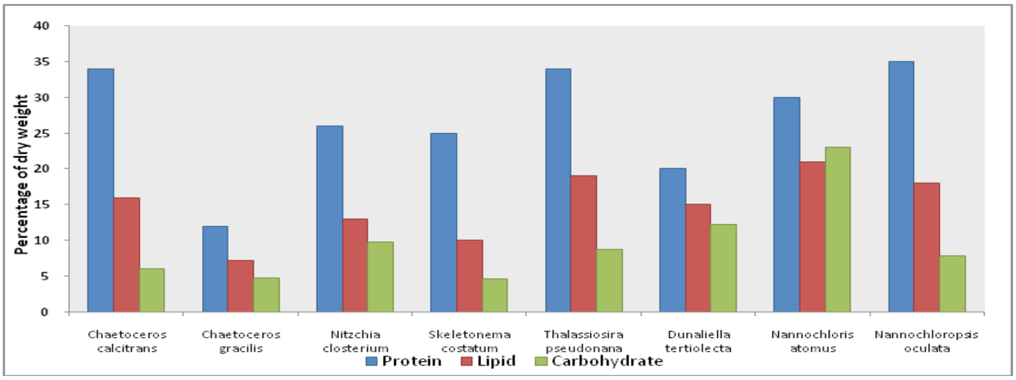

Fig 3. Concentrations of protein, lipid and carbohydrate in some species of micro-algae commonly used in aquaculture

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

WALNE'S MEDIUM FOR ALGAL CULTURES

Recommended for large volumes of aquaculture strains

Stocks		**per 100 ml**
	(1) Trace metal solution (TMS)	
	$ZnCl_2$	2.1 g
	$CoCl_2.6H_2O$	2.0 g
	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.9 g
	$CuSO_4.5H_2O$	2.0 g
	Make up to 100 ml with distilled water. This solution is normally cloudy. Acidify with a few drops of conc. HCl to give a clear solution.	
	(2) Vitamin solution	
	Vitamin B_{12}. (Cyanocobalamin)	10.0 mg
	Vitamin B_1 (Thiamine.HCl)	10.0 mg
	Vitamin H (Biotin)	200.0 µg
	Make up to 100 ml with distilled water.	
		per litre
	(3) Nutrient solution	
	$FeCl_3.6H_20$	1.3 g
	$MnCl_2.4H_20$	0.36 g
	H_3BO_3	33.6 g
	EDTA(Disodium salt)	45.0 g
	$NaH_2PO_4.2H_2O$	20.0 g
	$NaNO_3$	100.0 g
	TMS (1 above)	1.0 ml
	Make up to 1 litre with distilled water.	

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

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

f/2 Medium

Stocks	per litre
(1) $NaNO_3$	75g
(2) $NaH_2PO_4.2H_2O$	5.65g
(3) Trace elements (chelated)	
NA_2 EDTA	4.16 g
$FeCl_3.6H_2O$	3.15 g
$CuSO_4.5H_2O$	0.01 g
$ZnSO_4.7H_2O$	0.022 g
$CoCl_2.6H_2O$	0.01 g
$MnCl_2.4H_2O$	0.18 g
$Na_2MoO_4.2H_2O$	0.006 g
(4) Vitamin mix	
Cyanocobalamin (Vitamin B_{12})	0.0005 g
Thiamine HCl (Vitamin B_1)	0.1 g
Biotin	0.0005 g

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

* Add while stirring

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

✓ **Fertilizer used for outdoor microalgae culture**

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

✓ **Rotifers as live feeds**

Rotifers are also known as wheel animalcules due to its appearance. They are microscopic organism abundantly found in all the natural aquatic systems. It feeds on unicellular microalgae like *chlorella, Nannochlopsis, Tetraselmis, Isochrysis* etc. by movements of corona. In commercial finfish hatcheries rotifers are cultured on mass scale since they are the first feed of many of the finfish larvae due to their small and preferred feeding size (100 – 280 μ). Due to its high reproductive ability, mass culture can be achieved in few days. Rotifers are having ability to tolerate wide range of environmental conditions without affecting its growth and reproduction. Different kind of rotifers are being identified and classified based on their size, shapes of lorica and spines. Reproduction and growth of rotifers can vary due to rearing conditions like temperature and salinity. *Brachionus plicatilis* and *Brachionus rotundiformis* are the two species cultured in marine and brackishwater finfish hatcheries.

- **Types of rotifers:**

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

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

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

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

✓ **Pure culture of rotifer:**

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

✓ **Mass culture of rotifer**

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

✓ **Rotifer enrichment**

The nutritional quality of rotifers depends on their food source. Highly unsaturated fatty acids (HUFA) are essential for the survival and growth of the larvae. Rotifer feeds containing DHA and EPA can be valuable for marine and brackishwater fish larvae. Depending upon their food source, rotifers are composed of about 52-59% protein, up to 13% fat and 3.1% n3 HUFA. The high content of the essential fatty acid eicosapentaenoic acid (EPA 20:5n-3) and docosahexaenoic acid (DHA 22:6n-3) in some microalgae (e.g. 20:5n-3 in *Nannochloropsis occulata* and 22:6n-3 in *Isochrysis galbana*) have made them excellent live food diets for boosting the fatty acid content of the rotifers. The harvested / concentrated rotifers can be kept in these microalgae cultures for few hours for enrichment. The ratio of EPA:DHA can be manipulated by using different proportions of algae for enrichment. There are plenty of commercial enrichment media are available in the market for rotifers.

✓ **Artemia as live feed**

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

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

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

Table 1: Conditions required for artemia cyst hatching

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

✓ **Copepods as live feed**

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

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

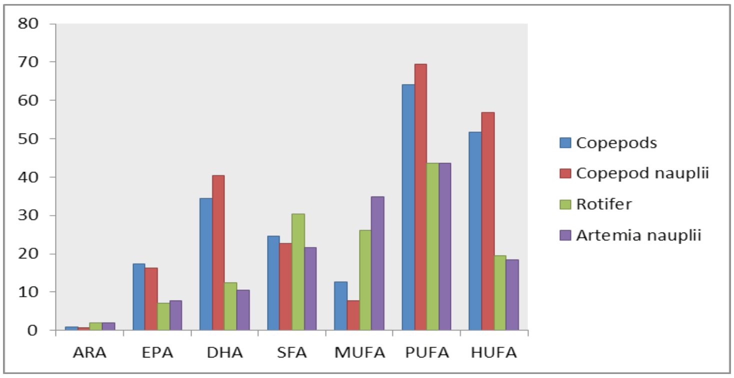

Fig 4. Fatty acid composition of various live feeds (Source: Meeren *et al*., 2008)

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

✓ Conclusion

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

✓ For further reading

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INDUCE BREEDING, SEED PRODUCTION AND LARVAL REARING OF MILKFISH (*CHANOS CHANOS)*

Aritra Bera, M.Kailasam , Babita Mandal, Krishna Sukumaran, M.Makesh, K.Ambasankar

Introduction

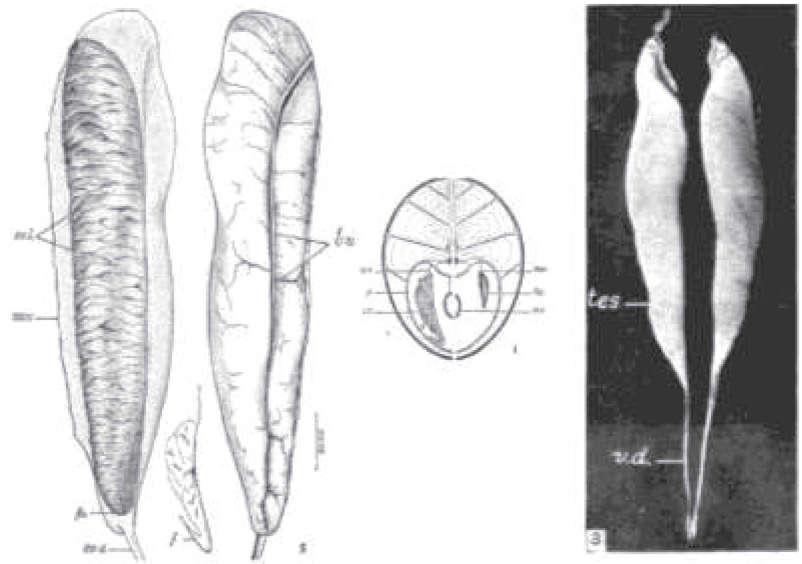
Milkfish (*Chanos chanos*) is traditionally cultured in Philippines. Indonesia also through its small scale hatcheries is a major producer of milk fish seeds. Milkfish culture is also practiced in some Pacific Islands viz. Kiribati, Nauru, Palau and the Cook Islands as well as in countries like Taiwan. There is increasing production from intensive mariculture cages now a days although most milkfish culture is undertaken in brackishwater ponds, low saline areas with extensive and semi-intensive way. The milkfish, *C. chanos*, does not reach gonadal maturity easily in captivity, although it achieves maturity under marine open sea cages. Since 80's artificial induction of milk fish spawning has been achieved in countries like Philippines, Taiwan and Hawaii and other small Asia-pacific islands in marine earthen / cemented ponds and sea cages.

Maturation, Induced breeding and larval rearing

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

Sexual Dimorphism

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



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

Maturity Determination

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

Problems and solutions related to milkfish maturation

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

- ❖ It has been seen, milkfish sperms can be stored for 10 days in 12.5 % DMSO at near zero temperature. As male fishes mature early than female cryopreservation of milt may be of great help. (Lam, 1984)

Brood Nutrition

A diet formulated by SEAFDEC/AQD containing 36% protein & 6% lipid and vitamin mixture to support sexual maturation and production of high quality eggs of milkfish is used to feed the broodstock. . With adult brine shrimp *A. salina* feeding milkfish showed reproductive readiness throughout the year in Isles Lagoon. (Lam, 1984*)*

Induction of Gonadal Maturation and Induced Breeding

Induced maturation of milkfish commonly starts at oocyte dia of 0.66 - 0.82 mm at 35 ppt salinity. Sex ratio of 2:1 (M:F) is better to maintain. Fertilized eggs are represented by 1.1-1.23 mm oocyte dia. Fish handling stress can be minimized by 2- phenoxy ethanol or diazepam as tranquilizers during handling. The hormones used can be Salmon or Carp pituitary homogenate in combination with HCG, or HCG alone. Hormone dosage can be variable: (Lam, 1984)

(a) SPH 6-10 mg/kg
(b) CPH 5-25 mg/kg
(c) HCG 180-2500 IU/kg

Numbers of hormone injections are generally 1-5 (mostly 2) with injection interval of 6-24 h (mostly 8-12 h). Time to stripping is commonly 6-17 h (12 h appears best) in case of oozing female and male. There are certain behavioral markers which may help to determine both whether the injection given is effective and also the time of stripping. These include the following (Lam, 1984):

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

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

Spawning

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

Larval Rearing

Feeding should initiate after 3rd day post hatching (dph). Critical period is between the 4th and 6th days. *Chlorella salina* added @ 3 – 4 x 10^5 cells/ml as feed for rotifer and to condition the water. Application of mix algae like *Isochrysis galbana* and *Tetraselmis chuii* shows *s*ignificant improvement in larval survival. Larvae at day 0, 14 and 21 day are highly euryhaline (0-70 ppt), those at day 7 are markedly stenohaline (27-28 ppt). This suggests that rearing milkfish larvae at a constant salinity of 27-28 ppt may improve their survival rate. (Lam, 1984). The hatchlings are stocked in the indoor larval rearing tanks (LRT) @ 30 no/l and first feeding initiated with rotifer *Brachionus plicatilis* from 2 day post hatch (dph). Rotifer density of 15 – 20 no/ml is ideal till 15 dph. *Artemia* nauplii was introduced along with the rotifer on 15 day post hatch (dph) @ 0.5 – 1.0 number/ml. The milkfish larvae reached to the early fry stage (20 mm) on 21 dph and accepts artificial feed .

Live Transport

Live transport of milkfish combines utilizing pre-transport starvation, anesthetic (2-phenoxy ethanol) at capture, chilled water during transport of brood stock.,. Live transport can be carried out in 4-13 year old broodstock placed in oxygenated transport plastic bags (2m long x 0.5 m wide) containing 40-L chilled (20-22°C) seawater and 5ml 2-phenoxyethanol (anesthetic). The transport bags were then placed in styrofoam/ thermocol boxes. Travel time should not exceed 6-7 hours to have fast recovery and no mortality. Eggs (embryonic stage) and newly-hatched larvae were also transported in oxygenated plastic bags containing 12-l seawater and supported by straw bags. Optimum density, temperature, and salinity should maintain are: 100,000- 120,000 eggs or larvae per bag, 28-30°C and 32-34 ppt of salinity.

Conclusion:

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

INDUCED BREEDING AND SEED PRODUCTION GREY MULLET (*MUGIL CEPHALUS*)

Krishna Sukumaran, Dani Thomas, M.U.Rekha, Pankaj Patil, Babita Mandal, K.Ambasankar, M. Kailasam

Introduction

Globally in the year 2014, the total production of grey mullets was recorded at 1,51,794 t of which 12,360 t was contributed by aquaculture production. Thus aquaculture made 8.14 % contribution to the total global production of the species. This is significantly low in comparison to the species like Asian seabass where 41.2 % contribution was made by aquaculture towards its total production. This is despite the fact that factors like good market value, growth rates, feeding niche and acceptance of artificial feed, adaptability to aquaculture systems, rearing environment and resistance to disease make grey mullet a highly favoured candidate for aquaculture. Given its position in food chain and its good market value, grey mullets have enormous potential for contributing to a sustainable aquaculture model which is the need of the coming decades. Hence, it is primarily the non-availability of adequate seeds which remains the primary constraint limiting the species from realising its true aquaculture potential.

Overview of the global status of grey mullet aquaculture and hatchery production

Currently, the largest contributors to the global aquaculture production of grey mullets is Egypt, followed by Republic of Korea, Italy, Taiwan province of China and Israel. Most of the global aquaculture production of grey mullet is still reliant on wild seeds. In India, wild seed availability of grey mullets is during the July to August in the west coast around Puduvypu region and around October-November in the east coast of India around Kakinada. Aquaculture of grey mullets in our country is on a limited scale in few pockets. However, degradation of nursery grounds and emerging restrictions on wild seed collection for aquaculture narrowing the chances of wild seed availability for aquaculture. It is in this context that broodstock development and standardisation of the induced breeding protocols are given paramount importance.

The history of induced breeding of grey mullet dates back to the 1960's in the pioneering work of Tang (1964). Full scale commercial hatchery production of grey mullet is not yet common. Induced spawning was achieved on an experimental and semi-continuous basis at Taiwan province of China, Hawaii, United States of America, Egypt, Abu Dhabi and India. Egypt is the largest producer of cultured grey mullet (over 90 % of the global production) was the first country to establish a mullet hatchery in 1990's producing few lakh fry but due to high production cost of the fry relatively to the locally sourced wild mullet the project was discontinued. Israel has made significant strides in captive reproduction of grey mullet and have a focus on developing all female populations. Yet as of now, most countries are producing grey mullets fry on a semi-continuous basis as a part of a multi-species hatchery or on experimental basis. Considering the pressure on wild seed resources, this scenario is bound change in the near future and the major source of mullet seeds will come from hatcheries.

Few factors that make grey mullet breeding relatively challenging

A few reasons why grey mullet captive seed production is considered relatively challenging is also linked to its reproductive characteristics. Grey mullets have a relatively confined reproductive season of few weeks. This also limits the window available for standardising breeding and larval rearing protocols in captivity. Besides, the species spawn only once every annual reproductive cycle and are termed single batch group synchronous species. Hence one female broodfish with mature oocytes is available only once in the season for successful intervention unlike multiple spawners. Relative to other species, the percentage of fish reaching functional maturity is also low and getting a good numbers of fish in the final stages of maturity is relatively lesser. This underlines the need for maintaining a relatively large broodstock a critical step for attaining success in grey mullet captive breeding. Presence of high level of inhibitory factors in captive reproduction of grey mullets is also reflected in the dosages of hormones used during induced spawning which is often more than three times of other brackishwater finfish species like Asian seabass. Grey mullet is cited as one of the classic examples of fish exhibiting dopamine inhibition along the reproductive axis. Dopamine acts as a feed back inhibitor against release of LH from the pituitary. Thus in fish where dopamine pathway is present, use of LHRHa for inducing final maturation may only limited success.

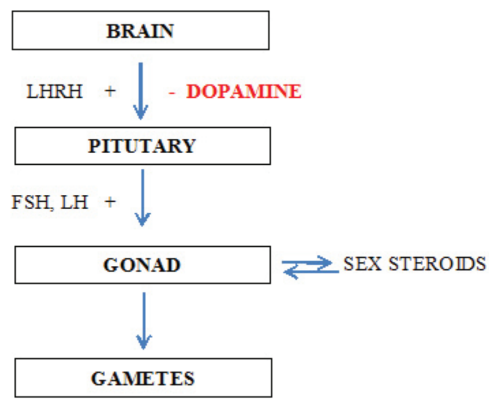


Fig. Dopamine- a predominant inhibitory factor governing the control of reproduction in grey mullets

Maturity assessment and readiness of fish for hormone treatments

Broodfish maintained in broodstock holding systems need to be assessed for the stage of maturity and onset of breeding season. Usually male fish encompasses the spawning season and period of maturity of the female, hence the appearance of milting males is a good indicator the breeding season. To assess maturity, the brood fish after being caught carefully and anaesthetised in 30 ppm 2-phenoxyethanol. The fish after being caught carefully and anaesthetised in 30 ppm 2-phenoxyethanol. Morphologically abdomen of the female appear to be relatively softer, the genital papillae are slightly prominent and the anal region slightly reddish and swollen. But confirmation of the maturity assessment is done through canulation to assess the ova diameter and the condition of the eggs. Reports suggest an ova diameter of 600 micro-m to be optimum for successful induced spawning in females. In case of males, gentle pressure is applied on the abdomen to check for milting two to three times. Males which express milting during the very first press is preferred for the induced trials.

Fig. a) Collection of canulated eggs for oocyte size assessment b) Pressing abdomen of male fish for checking expression of milt from male fish

Generally intramuscular injections are given to the selected brooders. The course of injection involves a preparatory or a priming injection followed by a decisive or a resolving after approximately 24 h. Even in a fish with fully mature egg the germinal vesicle is at the centre and this oocyte is incapable of being fertilised. Only when the conditions for final maturation are appropriate or on receiving the right hormone treatment will the germinal vesicle or the nucleus migrates to the periphery of the oocyte and disintegrates releasing the chromosomes into the cell final maturation.

Hormones used for final oocyte maturation for induced breeding in grey mullets

At the conclusion of vitellogenesis in oocytes, the final maturation of the oocytes is triggered by the action of the luteinising hormone (LH) on the ovarian follicles which then secrete the maturation inducing steroid (MIS). In grey mullet, the MIS is 17-α-20 β-dihydroxy-4pregnen-3-one. These act on the maturation promoting factors (MPF) located on the plasma membrane of the oocyte and initiates resumption of the meiosis and completion of oocyte maturation. During oocyte maturation the oocyte undergoes morphological changes like lipid droplet coalescence and yolk granule coalescence and this results in clearing of the cytoplasm, the germinal vesicle migration and breakdown at the periphery and hydration of oocyte leading to increase in final oocyte size and physiological changes leading to the production of a fertilisable egg.

Hormonal therapy used for induced breeding of grey mullet

Females selected based on the correct oocyte size are given a hormonal therapy to promote final oocyte maturation and ovulation. The most recommended hormonal therapy is the use of a priming dose of 20 mg kg^{-1} of carp pituitary homogenate and a resolving dose of LHRHa at 200 micro-g per kg^{-1} after 24 h via intra-muscular injections. Different authors have suggested varying hormone combinations.

Partially purified salmon gonadotropin, SG-G100 has been recommended at doses between 12-21 micro-g per kg body weight in inverse proportion to the egg diameter ranging above 600-700 micro-m. One third of the total dose is given initially followed by the remaining two-thirds after 48 h.

hCG- Priming dose of 20000 IU per kg followed by a resolving dose of 40000 IU per g after 24 h in fish with oocyte dia of 600 mcro-m (Kuo et al., 1973)

LHRHa- 300- 400 micro-g per kg body weight, one third as priming dose and two third as resolving dose after 24 h (Lee et al., 1987).

Many successful combinations were tried by Lee et al., (1988). A priming dose of HCG at 5000IU and a resolving dose of LHRHa at 200 micro-g per kg resulted in 100% spawning rate. However the best fertilisation rate of 66-86% was reported was using a combination of CPE and LHRHa at 20 mg kg^{-1} and 200 micro-g per kg respectively.

In Egypt a priming dose- 20-70 mg CPE or 10,000 IU HCG and a resolving dose of 200 micro-g LHRHa was reported for successful spawning and fertilisation by Gharabawy and Assem (2006).

A strong dopaminergic inhibition on reproduction is reported in grey mullets. This was clearly demonstrated in the works of Aizen et al., 2005. Using dopamine antagonists the dosage of GnRHa was reduced to priming dose- 10 micro-g per kg and resolving dose to 15 micro-g per kg for spawning of grey mullets.

OVULATION SPAWNING AND EGG COLLECTION

Ovulation is the release of egg into the ovarian lumen or the body cavity following rupture of the ovarian follicles that holds the oocyte. Following oocyte maturation, prostaglandins are secreted that stimulates ovulation. This release of the eggs spontaneously into the aquatic environment is called spawning.

After resolving dose administration, the broodfish are kept at a sex ratio of 2-3 males: 1 female in the breeding tank. A low stocking density and optimal conditions should be maintained to minimise stress and support natural spawning of the fish. The fish spawn 12- 14 h of receiving the resolving dose. The eggs are collected from the breeding tank and stocked into the incubation tanks. These eggs are about 880-900 micro-m in diameter. Approximately 1-2 million eggs are spawned by a fish of 1-2 kg size.

Sometimes, natural breeding behaviour followed by spontaneous spawning maynot be seen in captive conditions. In such cases gametes need to be artificially collected and fertilised through stripping. Stripping can be conducted only after ovulation, otherwise these gonads are termed green while stripping can be successfully done in ripe gonads. Both dry and wet stripping are practiced. Time of collection of oocyte after ovulation is important to ensure successful fertilisation. Stripping too early and too late affects the success of artificial fertilisation. Once eggs are released into ovarian cavity, they start to loose their viability, this is called over-ripening of eggs.

Eggs are stocked into the incubation tanks provided with sufficient aeration and gentle water flow. A salinity of 35 ppt ensures that the eggs are in suspension. The incubation period for mullet eggs is approximately 28-30 h at 26 ^{0}C. Salinities above 35% are advocated for good hatching rates.

Larval Rearing

A successful protocol for larval rearing is one of the final bottlenecks to captive seed production of grey mullet. Larvae are stocked at the rate of 10- 20 no per l in larval rearing tanks. Rotifers *Brachionus plicatilis* is advocated as the first feed and is provided at the rate of 10- 20 rotifers per ml. The rotifers are provided at the second day post-hatching. The background algae *Chlorella* or *Nanaochloropsis* is maintained at 5,00,000 cells per ml. By the 12th day post-hatching *Artemia* nauplii are gradually added at 1 no per l and this doubled every passing day. By the 20th day the larvae will be completely weaned to *Artemia naupii*. Artemia nauplii can still be fed at 20 no per ml distributed over three feedings. By the 17- 18th day, inert feed of 200- 300 micro-m size may be introduced. This method has been recommended to get a high survival rate for grey mullet larvae.

Further reading

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PRESENT STATUS OF MILKFISH *CHANOS CHANOS* FARMING

Kailasm M, , Pankaj Patil , Aritra Bera, Tanveer Hussain, Babita Mandal

Introduction

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

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

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

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

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

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

Indian scenario

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

Status of seed sources

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

Pen Culture:

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

Milkfish Culture in Cages.

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

Polyculture:

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

Extensive traditional farming

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

Intensive culture:

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

Semi-intensive Culture:

This method is characterized by smaller pond size of 1 to 5 hectares, at least 1 meter depth of water, and an increased stocking rate of 8,000 to 12,000 fingerlings per hectare in the rearing pond. Water exchange is enhanced by widening the gate, provision of separate drain gate and using water pump. Oxygen supply is improved by providing paddlewheel aerators and maintaining good phytoplankton growth later in the growing period. Natural food, mainly lab-lab, is grown and used as food in the first 45 to 60 days of culture in the grow-out ponds and commercial formulated diet with at least 27% protein is supplied thereafter. This method allows 2 to 3 crops and yields of up to 7.5 tons per ha per year.

PRESENT STATUS OF GREY MULLET *MUGIL CEPHALUS* FARMING

Pankaj Patil, Kailasm M, Krishna Sukumaran, Dani Thomas, M.U.Rekha, Tanveer Hussain

Introduction

The grey mullet (*Mugil cephalus*) is a euryhaline pelagic fish found near shores, it sometimes forages in lagoons, estuaries and lower courses of rivers and can tolerate freshwater. Juveniles feed on zooplankton, larger individuals filter algae, detritus, sediment and small invertebrates. Adults are found in coastal waters often entering estuaries and rivers sometimes far-up-river, lagoons and hypersaline environments. They commonly occur at water depths of 20 m but may be found offshore or in deeper waters.

They are mainly diurnal, feeding on detritus, micro-algae and benthic organisms. Flathead mullets are caught with beach seines, gillnets, cast nets, lift nets, barrier nets, trammel nets, and other artisanal gear. Some species occasionally swim far up river, while a few species spend their entire adult lives in rivers (Smith and Smith, 1986; Cardona, 2006).

Grey Mullet Aquaculture

Flathead mullet are an important aquaculture species, particularly in many Asian and Mediterranean countries (Fischer and Bianchi 2012) and has been in extensive and semi-intensive ponds. Mullets are usually grown or farmed for centuries in extensive, semi-intensive ponds and netted enclosures in shallow coastal waters of many countries. Mullet can be polycultured successfully with many other fish, including Common carp, Grass carp, Silver carp, Nile tilapia and Milkfish, and can be reared in fresh, brackish and marine waters.

Subsistence farming in ponds and enclosures has been traditional in the Mediterranean region, South East Asia, Taiwan Province of China, Japan and Hawaii.

Currently, Egypt is a leading country in mullet aquaculture. Most mullet aquaculture activities rely on the use of wild seed, e.g. Egypt (Saleh, 1991; Suloma and Ogata, 2006), Taiwan Province of China (Yeh, 1998), the Philippines, Italy (Landoli, 2000), Greece, Israel, Tunisia and Turkey (Sadek and Mires; 2000). In Egypt, from centuries flathead grey mullet has been traditional farmed in the hosha system in the delta region for centuries. However from 1960s, mullet has been cultured in semi-intensive ponds with tilapia and carps in Egypt. While in the Philippines, mullet has been raised mostly with milkfish. In Taiwan Province of China & Hong Kong, the *Mugil cephalus* is mostly cultured with carps in ponds. It has been reported that mullet have been farmed in India since ancient times; for example, it has been extensively cultured in Bengal, Madras and Kerala since 1947.

Commercial hatchery production of mullet seed is carried out in some countries. Induced spawning and production of fry has been achieved on an experimental and semi-commercial basis in the United States of America and Taiwan Province of China. The production of mullet fry on a limited scale for aquaculture has been reported in Italy, Israel and Egypt (Saleh, 2006). The development of hatchery production techniques are only practiced commercially for flathead grey mullet as the techniques for seed production of other important species (e.g. thinlip grey mullet) are not yet developed.

Indian scenario:

India has a long coastline of 8,129 km extending over nine maritime states and the island EEZ area of 2.02 million km^2 and a shelf of 0.512 million km^2 that makes it possible for one of the richest multispecies fisheries in the world. In addition to the vast coastline, it has more than 8.5 million ha of derelict inland saline area. But yet India's mariculture progress has been very slow. The slow progress is due to the collapse of the shrimp farming industry because of environmental concern and disease problems. Although the present attention is towards diversification of fish species other than shrimp, the commercial venture are constrained due to unreliable wild seeds and lack of technology for commercial marine finfish hatchery seed production.

In India, grey mullet is cultured in estuaries and ponds, and is much relished for its flesh quality and good flavour. Recently, mullets (family Mugilidae) have the mainstay of finfish culture in coastal & estuarine impoundments (Pokkali Fileds). The traditional and semi-intensive pond farming mainly depend on availability of seeds. It is commonly available on the east and west coasts of India. The fry of *M. cephalus* migrate into estuaries of south-east and south-west coasts immediately after onset of south-west monsoons in November–April (Curian, 1975) and north-east coast in January–March and July–August. Full-scale commercial production of *Mugil cephalus* is a new area of aquaculture diversification in India. It is an economically important euryhaline and eurythermal species contributing to sizable fisheries of estuarine and coastal regions in many countries including

During the late summer months, adults migrate to the sea in large aggregations to spawn. Fecundity is estimated as 0.5– 2.0 million eggs per female, depending upon the adult size. Hatching occurs about 48 hours after fertilization, releasing larvae approximately 2.4 mm long. When the larvae are 16–20 mm, they migrate to inshore waters and estuaries, Shoals of fry are collected by fine seine nets, transported in seawater to hapas or shore and transported to nursery units, or nursery facilities in grow-out farms.

Under extensive brackish water pond culture conditions, the production average 2.0-2.5 tonnes/ha/year during 8-9 months grow-out period. Intensive farming restricted due to non-availability of hatchery seed. If mullet are mono cultured, manuring may be sufficient to reach the required feed level. Acclimatized to the appropriate salinity and stocked as 10–15 g individuals at about 6 200–7 400/ha, a harvest of 4.3–5.6/tonnes/ha/crop can be obtained. Similalry, in semi-intensive polyculture with tilapia and carp, mullet fingerlings are stocked at about 2 500–3 700/ha together with 1 900–2 500/ha of 100 g common carp juveniles and about 62 000–74 000/ha of 10–15 g Nile tilapia fingerlings. Total harvests are typically 20–30 tonnes/ha/crop of which 2–3 tonnes are mullets. After an on-growing season of 7–8 months in the subtropical region, flathead grey mullet reach an individual weight of 0.75–1 kilogram. Mullet grown for two successive seasons, reaches 1.5–1.75 kilograms. The choice of rearing period and technique depends on market demand and economics.

Pen culture method would be a more reliable and profitable one for the culture of Grey mullets. But for the fencing to prevent escape of the stocks and limiting predation by predatory organisms from outside, they are reared in their own natural habitat itself. Hence, there is every possibility to expect fast growth and high production, when supplemental feeding is also done.

Feed

In monoculture, mullet feeds on natural food and on the by-products of grain mills and rice polishing plants. In polyculture, manufactured extruded pellets are produced either in feed mills specialized in the production of fish feed or, in many cases, in chicken feed mills that have a line for fish feed production.

Experimental trials

A number of experiments on monoculture of polyculture culture of grey mullet is done by ICAR-CMFRI & ICAR-CIBA. Significant achievements were made in the coastal pond culture of milkfish & grey mullets at Tuticorin-polyculture of *Chanos chanos*, *Mugil cephalus* and *Penaeus indicus*; polyculture of milk fish, mullets, carps and prawn at Sunderbans and monoculture experiments with Grey mullets, *Liza waigiensis* and Valamugil seheli at Mandapam, Culture of *Mugil cephalus* at different densities in pond culture at Kakdwip, etc. Pen culture experiment carried out at Mandapam and Tuticorin with milkfish & grey mullets at a stocking density of 50,000/ha. However, still the mullet fish farming in India is in infant stage due to the lack of commercial scale availability of hatchery produced seed is the major bottleneck for any large scale venture of grey mullet fish. The availability of seeds from wild is often unpredictable & hence farming based on wild collected seed not be a sustainable venture. Hence, the development & standardization of seed production of grey mullet should receive research priority. It is felt that the establishment of commercial hatcheries for ready supply of seed is the primary step for the development and expansion of mullet fish farming in India

NURSERY REARING OF MILKFISH (*CHANOS CHANOS*) AND GREY MULLET (*MUGIL CEPHALUS*)

Tanveer Hussain, Gouranga Biswas, Aritra Bera, Dani Thomas, Pankaj Patil, Raja Babu

Status of seed sources

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

Site selection

Existing brackish water fish farms that are fully developed and operational can be used for milkfish farming. The site should have a minimum water depth of 0.8 - 1 meter; good quality of water with optimal salinity of 10 - 30 ppt, Temperature of 26 - 30° C, Water pH of 7.5 - 8.5, Dissolved oxygen of 3.5 – 5 ppm around the year. Milkfish can be grown in freshwater and can tolerate low levels of DO and high levels of Ammonia. Pond soil should be sandy / silty clay loam. Good access to roads and power supply is also necessary for milkfish farming site to reach markets for better revenue generation.

Pond preparation and lab-lab (benthic algae) production

Pond preparation such as complete pond draining and drying, soil sealing, pond leveling and repair, predator eradication, liming and tilling should be carried out before starting milkfish farming. Milkfish Nursery pond with lab-lab

- ✓ Application of organic/inorganic fertilizer in the pond water for the stimulated growth of natural food organisms.
- ✓ Application of chicken manure at the rate of 2 tons/ha followed by water level increment up to 5 cm is recommended for the purpose of initial manuring.
- ✓ Application of urea at the rate of 15 kg/ha after 2-3 days is necessary for the breakdown of already applied chicken manure
- ✓ Water depth should be increased 3-5 cm gradually over a period of 15 – 30 days to make final water depth of 0.8 – 1 meter for stocking of milkfish fry in pond.
- ✓ Abundant growth of natural food (lab-lab) can be achieved by extended pond preparation of 45 days for better nutrition of milkfish fry.

Nursery rearing

- ✓ Milkfish seed of 1-2 cm (fry) can be stocked at a density of up to 20- 30 no/ m² (2-3 lakhs/ha) and are allowed to feed on naturally-grown microbenthic food known as 'lablab'or benthic algal mat.
- ✓ Urea at the rate of 15 kg/ha can be applied every 7-10 days to maintain good growth of natural food.
- ✓ Pond water should not be freshened / released for at least 3 days after fertilization.
- ✓ Nursery rearing can be carried out in hapa type suspended nylon nets in brackish water ponds or lagoons.
- ✓ Artificial feeds such as rice bran, corn bran or formulated feeds can be provided with natural food.
- ✓ Milkfish fry grows to 5 – 8 cm (fingerling) after 4-6 weeks of rearing in nursery pond which can be cultured in grow out ponds/pens.
- ✓ Fingerlings should be harvested using drag nets made of knotless nylon net or mosquito net to avoid any damage to the fish.

Transition / Stunting ponds

Milkfish fingerlings can be stocked at the rate of 15 fingerlings/ m² in transition pond for 6 – 12 months for stunted milkfish production with natural food of lablab and lumut (filamentous algae). Occasional feeding of rice bran at the rate of 5 % of body weight can be done.

- ✓ After 1 year of stunting in transition pond, they attain weight of 35-50 g

  with 50 – 60% survival. Milkfish from transition pond
- ✓ Fingerlings of 5 g to 150 g body weight are used as tuna bait in tuna fishing sector due to its active movement and shiny appearance.
- ✓ Bigger sized fingerlings of 40–80 g body weight are preferred for culture in pens and floating net cages.

Nursery rearing of Grey Mullet (*Mugil cephalus*)

Among 70 valid species of mullets belonging to 20 genera under the family Mugilidae, the species having highest growth is widely called as striped/ flathead grey mullet, *Mugil cephalus*. This species has a cosmopolitan distribution between latitude 40°N and 40°S covering all the oceans. It is an economically important euryhaline and eurythermal species contributing to a sizable fishery of estuarine and coastal regions of countries like China, Egypt, India, Israel, Italy, New Zealand, Nigeria, Sri Lanka, Taiwan and Tunisia. Owing to omnivorous feed habit of grazing on plant detritus and microflora, it is an ecologically important species feeding at the lowest trophic level and suitable for mono or polyculture. To the world mariculture production, a substantial contribution is shared by striped/ flathead grey mullet as one of the species.

In India, *M. cephalus* is a much relished for its flesh quality and good flavour and farmed scientifically in brackishwater ponds and impoundments. However, traditional and semi-intensive pond framings mainly depend on availability of seeds. Fry of *M. cephalus* migrate into estuaries of south-east and south-west coasts immediately after onset of south-west monsoons in November-April and north-east coast in January-March and July-August. The small fry (15-25 mm) are not suitable for direct stocking in grow-out ponds. Since the growth of this fish is slow during early life stage, it is desirable to conduct a pre-stocking seed rearing to obtain bigger size individuals suitable for grow-out culture. In culture ponds, the grey mullet accepts artificial feed in presence of natural food organisms. Pond fertilization using organic and inorganic manures affects the growth of grey mullet. Therefore, utilizing the natural pond productivity and employing other interventions grey mullet seeds are reared for production of stockable size fingerlings.

Seed rearing of grey mullet

Among different seed rearing methods, such as only fertilization or feeding, combined fertilization-feeding, fertilization-compost application and fertilization-periphyton systems, the best performances of fish can be obtained in the combined fertilization-feeding and fertilization-periphyton rearing systems.

Low density fertilization-feeding system

After treatment of pond bottom with lime, water is taken and fertilized with cattle manure, urea and single super phosphate at 500, 30 and 30 kg/ha, respectively. After 7 days of fertilization, ponds are stocked with *M. cephalus* fry (0.55 g/ 36.0 mm) at 15000 nos./ha. Formulated feed prepared from locally available ingredients (mustard cake, rice bran, wheat flour, fishmeal etc.) is provided as supplementary feed @ 20 to 5% of body weight. Ponds are fertilized fortnightly with the above mentioned fertilization materials at the same dose. Liming is done at fortnightly intervals with lime stone powder at 250 kg/ha. After 150 days of rearing, grey mullet attains average body weight (ABW) of 96 g.

High density fertilization-periphyton system

After bottom treatment followed by water taking according to the method mentioned earlier, ponds are fertilized with mustard cake, urea and single super phosphate at 200, 20 and 20 kg/ha, respectively. After 6 days, bamboo poles are erected vertically in the pond to cover 10% of pond surface area as substrate for periphyton growth. After 10 days of bamboo pole fixing, pond is stocked with *M. cephalus* advanced fry (3.36 g/ 63.7 mm) @ 30000 nos./ha. During rearing, all the ponds are fertilized fortnightly with mustard cake at 100 kg/ha. Agricultural lime at 100 kg/ha is applied one day before fertilization throughout the rearing period. Grey mullet fingerlings attain ABW of 28 g in 120 days of rearing.

Application of Biofloc technology in high density nursery rearing of milkfish and mullet

The environmental friendly aquaculture system called “Biofloc Technology (BFT)” is considered as an efficient alternative system since nutrients could be continuously recycled and reused. The sustainable approach of such system is based on growth of microorganism in the culture medium, benefited by the minimum or zero water exchange. These microorganisms (biofloc) has two major roles: (i) maintenance of water quality, by the uptake of nitrogen compounds generating “in situ” microbial protein; and (ii) nutrition, increasing culture feasibility by reducing feed conversion ratio and a decrease of feed costs. Nursery rearing of milkfish and mullet can be done in biofloc based high density fry rearing using different cost-effective carbon sources. The particulate organic matter and other organisms in the microbial food web have been proposed as potential food sources for aquatic animals. In BFT, microorganisms present a key role in nutrition of cultured animals.

The macroaggregates (biofloc) is a rich protein lipid natural source available "in situ" 24 hours per day. In the water column occurs a complex interaction between organic matter, physical substrate and large range of microorganisms such as phytoplankton, free and attached bacteria, aggregates of particulate organic matter and grazers, such as rotifers, ciliates and flagellates protozoa and copepods, forms ideal food for milkfish/ mullet fry. The consumption of biofloc by milkfish/mullet can improve the growth rate, decrease of FCR and associated costs in feed. Growth enhancement has been attributed to both bacterial and algae nutritional components, which up to 30% of conventional feeding ration can be lowered due to biofloc consumption in fish.

BRAKISHWATER ORNAMENTAL FISH BREEDING AND CULTURE

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Ornamental industry is at present a multi-billion dollar industry with an estimated value of 15 billion US dollars in which 1,500-1,600 species are traded globally (Moorhead and Zeng, 2010; Oliver, 2001). The major chunk of the ornamental fish traded constitute of the freshwater fish (almost 90 percent), however in terms of value their marine counterparts contribute significantly higher. A notable difference is that the freshwater species are mostly captive bred and that the marine species are collected from wild, 90- 95% (Oliver, 2001). The USA, Europe and Japan are the largest international markets for ornamental fish trade; however, Asia is home to more than 65% of the exports (Ghosh et al., 2003). Singapore has been a consistent leader in ornamental fish exports, followed by Indonesia, Malaysia and China. In India, Kolkatta has emerged as the major hub for ornamental trade accounting to almost 90% of the exports followed by Mumbai and Chennai. The existing scenario of export market based on wild collection is not a healthy one. Efforts in developing and propagating seed production of untapped indigenous species will go a long way for developing a robust ornamental fish industry in India. In this regards, CIBA has placed a major thrust on developing seed production technologies of many commercially important species. Along with developing ornamental fish culture as large scale production models, CIBA places a major emphasis on developing brackishwater ornamental fish as a livelihood option. Presently the important candidate species on which CIBA places a major thrust are Spotted Scat, *Scatophagus argus*, Silver Moony fish, *Monodactylus argenteus*, Green Chromide *Etroplus suratensis*, Orange chromide, *Etroplus maculatus*, Banded Chromide, *Etroplus canrensis* and Crescent perch, *Terapon jarbua* are some of them, however, there also lies great opportunity to do so in a sustainable manner by investing in research for development of captive seed production technology for these brackishwater species.

Breeding aquarium fish is one of the steps to becoming a skilled aquarist. In order to breed a species, the aquarist usually needs to be able to distinguish between the sexes and to be able to recreate natural conditions to stimulate spawning process.

There are many steps (techniques) to be followed to breed an aquarium fishes such as follows:

I. Fish Sex Determination:

Determining the sex of a fish is an important step in knowing whether one has a pair. Most fishes can be categorized as sexually dimorphic or sexually isomorphic. In sexually dimorphic species, the sexes can be easily distinguished by primary (shape of sex organs) and secondary differences (size, shape, color). Male's fishes are frequently more colorful, larger, and have more elaborate finnage. Among the more brilliant outstanding of sexual dimorphism can be found in Cichlids, Killifish, and Livebearers. In sexually isomorphic species, there are minute, if any, apparent sexual differences. Often, the only way to distinguish between the sexes is the shape of the genital papilla, which is only visible around spawning times. In some isomorphic species, the males are slightly larger and the females are slightly rounder in the belly. Some sexually isomorphic species have no known external sexual differences.

II. Selection of Brooders/Parent Fishes:

Once males and females have been distinguished, a suitable pair or spawning group should be chosen. There are several important traits to seek in choosing the parent fish. Choosing fish that display good markings and color that should produce attractive young fries and fingerlings. Use mature, healthy compatible fish for spawning because unhealthy fish, if they spawn, may produce unhealthy or deformed young fishes.

III. Reproductive Strategies:

A. Egg-layers Fishes:

The majority of aquarium fish are egg-layers with external fertilization. Egg-layers can be divided into five groups: egg-scatterers, egg-depositors, egg-burriers, mouth-brooders, and nest-builders.

Egg-scatterers: These species simply scatter their adhesive or non-adhesive eggs to fall to the substrate, into plants, or float to the surface. These species do not look after their brood and even eat their own eggs. These, often schooling, fish may spawn in groups or in pairs. Example: Egg scatterers with non-adhesive eggs, Zebra Fish, *Danio rerio* and Egg scatterers with adhesive eggs Gold fish, *Carassius auratus* submerged Hydrilla plants are used as substrate to attach adhesive eggs.

Egg-depositors: These species deposit their eggs on a substrate (tank glass, wood, rocks, plants). Egg depositors usually lay fewer eggs than egg-scatterers, although the eggs are larger. Egg-depositors fall into two groups: those that care for their eggs, and those that do not. Among eggs depositors that care for their eggs are Cichlids and some Catfish. Egg-depositors that care for their young can be divided into two groups: Cavity spawners and Open spawners. Cavity spawners lay their eggs in a cave, while open (shelter) spawners lay their eggs on an open surface. These fish form pairs and have advanced brood care where the eggs are defended and cleaned. The eggs take a few days to hatch, and the fry are often guarded by the parents. Various Catfish, Cyprinds, and Killifish make up the majority of egg-depositors that do not care for their young. These species lays their eggs against a surface, where the eggs are abandoned. These species do not usually eat their eggs. Examples Barbs, *Rasbora sp.*

Egg-burriers: These species usually inhabit waters that dry up at some time of the year. The majority of egg burriers are annual Killifish,(*Aplochelius sp.*) which lay their eggs in mud. The parents mature very quickly and lay their eggs before dying when the water dries up or in drought condition. The eggs remain in a dormant stage until rains stimulate hatching. They grow up to 3-4cm total length and are short lived.

Mouth-brooders: Mouth-brooding, also known as oral incubation and buccal incubation, is the care given by some groups of animals to their offspring by holding them in the mouth of the parent for extended periods of time. Paternal mouthbrooders include the arowana (*Osteoglossum bicirrhosum*), Betta, *Betta pugnax*, and Sea catfish, *Ariopsis felis*, *Arius sp.*, Tilapia, *Sarotherodon melanotheron,* and Snake head*, Channa striatus.* Fish species that carry their eggs or larvae in their mouth. Mouth brooders can be broken up into ovophiles and larvophiles. Ovophile or egg-loving mouth-brooders lay their eggs in a pit, which are sucked up into the mouth of the female.

Nest-builders: Nest builders build some sort of nest for their eggs. The nest is usually in the form of bubble-nest formed with plant debris and saliva-coated bubbles (Siamese –fighter fish, Angel fish, Labyrinth fish, Catfish), or an excavated pit in the substrate (Cichlids). Nest builders practice brood care.

B. Livebearers

Livebearers are fish that bear live young. There are two types of livebearers: ovoviviparous, where the eggs form and hatch within the female before birth; and viviparous, where no eggs are formed, and the young are nourished through an umbilical-like cord or from secretions by the female. Livebearers are often prolific, easily bred species. One of the best ways to induce fish to spawn, especially difficult-to-spawn species, is to simulate natural conditions. Examples are Guppy, Platy, Mollies etc. Among factors that encourage fish to spawn are the environment, the food, and the rainy season.

Environmental Parameters: The right water conditions are among the most basic requirements in spawning of fishes. Thus the water conditions should be similar to those in the natural environment of the species. By following the suggestions under "breeding" or "water" in the species descriptions, approximate natural water conditions can be found. Another important environmental condition is the right tank set-up including hiding places, spawning sites, and lighting, water current and social conditions.

Food: The right foods are important to encouraging spawning. Without proper foods, natural conditions cannot be entirely recreated. Some of the live foods that often can make a difference in spawning success are mosquito larvae and fruit flies.

Monsoon Rainy Season: Many fish species spawn during the rainy season in nature. By simulating the rainy season in aquaria, difficult-to-spawn species can be induced to spawn. Rains affect the water chemistry, the water height, and the water temperature also.

Target Conditioned Fish: Paired Fish/Target fish can be used to help strengthen the bond between a fish pair. Target fish can be another of the same species or a similar species that is placed in the tank with the breeding pair. This third fish will serve as an object of the aggression of the pair. The pair will work together to chase off the target fish and not fight between themselves. Only use the target fish method in a large tank with plenty of hiding places, so that the target fish is not harmed. PVC Pipes can be used as hide outs in the breeding tanks. There are many brackishwater ornamental fishes such as follows:

I) Orange chromide, *Etroplus maculatus*

Taxonomy Position

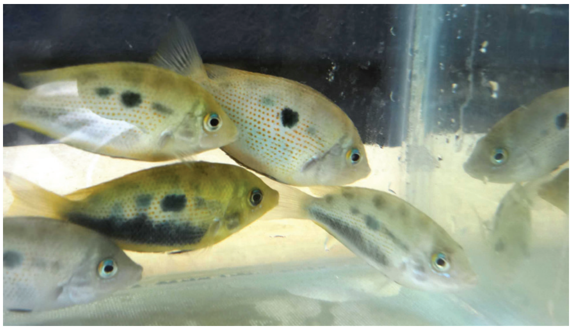
Class- Actinopterygii

Order- Perciformes

Family- Cichlidae

Genus- *Etroplus*

Species- *maculatus*



The Orange chromide is a species of fish endemic to freshwater and brackish streams, lagoons and estuaries in southern India (Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu,) and Sri Lanka. It is also known as pallathi in Malayalam and Pitalanga kachi in Tamil. This species is well known for its parenting behaviour. The fish is an omnivore feeding on zooplankton and filamentous algae. It attains a maximum total length of 80 mm. Fecundity of the fish is 1378 (Jayaprakash et al., 1979) approximately and reported to be between 140- 231 eggs per spawning (Bindu and Padmakumar, 2012). Size of the fishes varied from 6-10g in weight and 6-9 cm length. In nature the fish form a breeding pairs and attach eggs on the substrate. The species exhibits parental care and the offsprings are taken care of by the parents. The orange chromide, *Etroplus maculatus* has attractive colour pattern with the presence of elongated oval shaped body. It has yellow to bright orange colour with three short transverse bars located mid section of the body. The fish has large black area in the lower section of the body with large black coloured eye.

Breeding and Juvenile Production of Orange Chromide, *E. maculatus* in backyard

Presently, *E. maculatus* is being collected from the natural water bodies and sold in aquarium shops with price ranged from Rs.10-15/fish. Wild collection of *E. maculatus* is seasonal and inadequate which would not meet the increasing demand from the traders. Therefore, developing compressive technology package for breeding of *E. maculatus* would help to produce large scale juveniles for continuous supply. In this context, an attempt was made on breeding and juvenile production of *E. maculatus* under controlled condition in FRP tank in backyard.

In each tank, two pairs of orange chromide (L:7.2-8.5cm/W: 7.0-11.0 g) of male and female (2:1) were stocked and maintained with the water quality parameters of salinity: 5-10ppt, temperature: 25-28^{o}c, pH: 7-5-8.2 and dissolved oxygen: 6.0-8.0 ppm. Spawning could be observed after 10-12 days of stocking in the broodstock tank. Rigglers stage larvae (3 days post hatch) of 250-300 numbers could be collected from each tank. Repeated spawning could be observed between 10-15 days after rigglers collection. Average Fecundity was 292±109.38 and Spawning frequency interval was 12 ± 2.13 days. The eggs were pale yellow coloured, ellipsoidal in shape, with an average size of 1.5 mm, attached to the nest surface (Earthen pot) by a stalk. Egg incubation period 46-48 hr with av. 48 hr. Larvae were stocked @ 5 nos./l in 100 liter FRP tanks and rearing continued up to 75 days. Rotifers and *C. salina* were supplied together as feed up to 15 dph followed by *Artemia* nauplii up to 25 dph and afterwards the fishes were weaned to formulated artificial diet. The *E. maculatus* juvenile attained mean length of 4.50 ± 0.54 cm and mean body weight of 2.2 ± 0.43 g at 75 dph. The present study findings have provided immense scope to develop breeding model of *E. maculatus* as livelihood options for small scale farmers and self help group. A business module has been developed and a monthly income of Rs.4000-5000/- could be generated through rearing of orange chromide fishes in backyard.

II) Banded Chromide , *Etroplus canarensis*

<u>Taxonomy Position</u>

Class- Actinopterygii

Order- Perciformes

Family- Cichlidae

Genus- *Etroplus*

Species- *canarensis*

Canara pearlspot or Banded chromide, *Etroplus canarensis*, is a species of cichlid endemic to South Karnataka in India (only from the Kumaradhara-Netravati river system). Its habitats are highly-seasonal in nature with annual monsoons bringing about severe increases in water depth, flow-rate and turbidity. The substrate was mainly composed of small rocks and leaf litter with some tree roots projecting into the water along one margin. Temperature: 22 – 32 °C; pH: 6.0 – 7.5, Hardness: 18 – 179 ppm. The Maximum standard length is 100 – 110 mm. Omnivorous in feeding habits, Sexual maturity appears to be reached at around 2 years of age. In Rivers the fish are thought to breed during the months of December and January, when temperatures are cooler and high monsoon waters have receded, and simulation of the change between these seasons can sometimes induce captive spawning process.

Sexual Dimorphism: There are no obvious external sex differences in fin length or intensity of colour pattern. Adult males are larger and heavier than female fishes. It belongs to handful of species of Cichlids from Asia, amphidromous in migration patterns; this is listed as "Endangered" in The IUCN Red List of Threatened Species" 2013.2. It has been assessed as Endangered because of its restricted distribution to two locations (Netravathi & Kumardhara River) in India. Matured ova of *E. canarensis* (~ 430 micron sizes) were observed in females maintained at Muttukadu Experimental Station, (MES), Muttukadu.

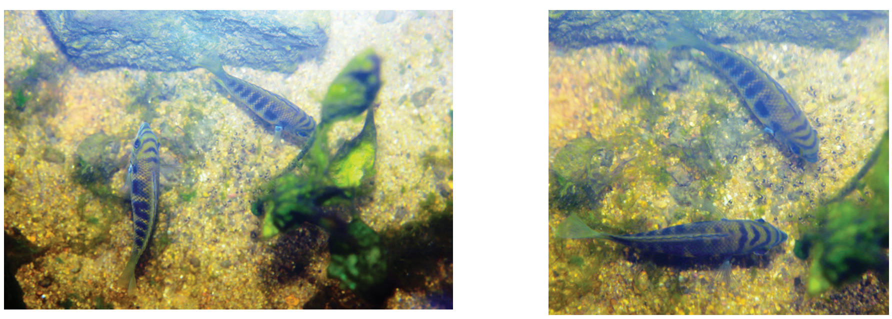


Figure.1. Induced captive natural spawning of Canara Pearlspot, *Etroplus canarensis* was achieved in 2nd October, 2016 at MES, Muttukadu, CIBA, Chennai.

In Indian River it breeds during December and January, when water temperatures are cooler and high monsoon waters have receded, and simulation of the change between these seasons can sometimes induce captive spawning behavior. Canara Pearlspot lay eggs on a substrate (substrate spawner) are biparental. Both the male and the female care for the filamentous stalked attached eggs. Parents tirelessly fan the eggs with movements of their pectoral fins to provide them with a good supply of oxygen. They clean the eggs by brushing them with their pectoral fins. With their mouth they remove dead or diseased eggs from the bunch. Eggs are normally deposited in a sheltered position on the side or top of a rock to which they are attached by short filaments. Fertilized eggs hatch after 4 days at temperature of about 28-30 ^{C}; fry are free swimming in further 2-3 days with shoaling behavior.

Larval Rearing Protocol:

Twelve days old larvae were separated from parents and stocked @ 5 nos. /l in 100 liter FRP tanks and rearing continued upto 60 days. Rotifers, *Brachionus plicatilis*, green algae, *Chlorella salina* and *Artemia* nauplii were supplied a live feeds to the larvae. Rotifers and green algae were supplied together upto 15 days of post hatch followed by *Artemia* nauplii upto 30 dph and afterwards the fishes were weaned to formulated artificial diet. Water quality parameters of spawned tanks were ranged from pH: 7.30-8.12, Temperature: 27-30^{C}, Total hardness: 262.6 – 333.3 ppm, Ammonia: 0.05-0.06ppm, Total Alkalinity: 82.72 101.52 ppm. Omnivorous in feeding habits and it has been observed that it used to graze on algae that are attached to rocks in the wild. Hence a varied diet with algae is recommended.

III) Crescent perch /Target Fish, *Terapon jarbua*

Taxonomy Position

Class- Actinopterygii

Order- Perciformes

Family- Teraponidae

Genus- *Terapon*

Species- *jarbua*

A new candidate species for brackishwater ornamental/food fish Terapon jarbua commonly called as Target Fish, Crescent Bass, Crescent Perch or Tiger Bass. This species is euryhaline in nature and can tolerate fresh to seawater salinity and could be a potential candidate species in brackish and freshwater aquaculture for food and also ornamental purposes. Breeding and seed production is one of the major constrain in aquaculture of this fish, hence a breeding trail was attempted for seed production of this fish under captive condition. Mature male and female was collected from brackishwater pond and acclimatized to captive condition for a week under flow through system (Temp: 25-260C; Salinity: 27 ppt). To assess the maturity, female fishes were cannulated and male were gently pressed near the vent. Female having the oocyte diameter above 460µ were selected along with the oozing male. Four breeding set was arranged, and in each set 2-male and one female was introduced. From the four set two set were administered with HCG@300 \IU/Kg and other two set were with LHRHa @ 75µg/Kg, half the dose was given to male. After 36 h of post injection all set was spawned (fertilized embryo size: 750 µ) and embryo was semi-buoyant in nature, and after 16-18 h of incubation hatching was observed. Sizes of hatchlings were 2 mm with the yolk sac length of 75µ and a single oil globule. After 12 h of post hatching concentrated algae (*Chlorella* sp.) were introduced in the larval rearing tanks @ 20 lit/400 lit and after 48 h rotifer feeding was started. A total of more than 3 lakhs spawns (2 days old) were collected and transferred to nursery rearing protocol. CIBA has successfully initiated induced breeding trials with hormonal manipulation using HCG and LHRH (CIBA ANNUAL REPORT, 2014-15).

Conclusion:

India's share in ornamental fish trade is estimated to be less than 1 % of the global trade. The major part of the export trade is based on wild collection. There is very good domestic market too, which is mainly based on domestically bred exotic species. The overall domestic trade in this field cross 1000 lakh and is reportedly growing at the rate of 20 per cent annum. The earning potential of this sector has hardly been understood and the same is not being exploited in a technology driven manner. Considering the relatively simple techniques involved, this activity has the potential to create substantial employment opportunities, besides earning foreign exchange also.

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HEALTH MANAGEMENT IN BRACKISHWATER FINFISHES

M. Makesh, Krishna Sukumaran, Tanveer Hussain, M.Kailasam

Health management in fish hatcheries and grow-out culture is essential for the successful production of fish seeds and table size fish. The quality and quantity of seeds produced is a yardstick to judge the success of a hatchery and the quality of seeds produced depends on health management measures adopted by the hatchery. In a hatchery, larvae are often stocked in high densities and hence poor management will result in weak seeds with low survivability. Prevention is considered as the first step in controlling infectious diseases in hatcheries. Simple measures like sanitizing hands before handling fish, having foot dips with disinfectants at all entry points in the hatchery, disinfecting the source water etc. can help a lot in controlling infections. The following health management techniques need to be adhered to in hatcheries to maintain and produce healthy disease free seeds.

1. Quarantine: All brooders brought to the hatchery need to be quarantined till it is tested and found to be free of any diseases or parasites. The quarantine tanks should ideally be placed away from the hatchery operations. Brooders brought to the hatchery should be acclimatized in the quarantine tanks and clinically examined for the presence of ectoparasites, lesions, fouling of gills etc. Samples should also be collected and tested for the presence of subclinical infections. Once the stock is found to be healthy and free of infections they may be transferred to broodstock tanks.
2. Biosecurity: Biosecurity is critical for the successful operation of the hatchery. Strict biosecurity measures need to be adopted to prevent the possible entry of pathogens into the hatchery. The following measures need to be adopted in a fish hatchery.
    a. Ideally the hatchery should be located away from general public and the entry to the hatchery should be restricted to authorised personnel only.
    b. Visitors should be asked to follow the guidelines strictly while visiting the hatchery.
    c. All vehicles entering the hatchery should pass through a disinfectant pit so that the entire tire of the vehicle comes in contact with the disinfectant before the vehicle enters the hatchery.
    d. The source water should be filtered with sand filters to prevent entry of weed fishes which may carry pathogens. The source water should also be disinfected to get rid of pathogens by UV treatment or ozonisation.
    e. Each building /culture area must be provided with foot dips containing potassium permanganate.
    f. The hatchery personnel need to sanitize their hands before any hatchery operation.
    g. Bird proofing, rodent and stray animal control measures should be adopted.
3. Use separate tools like hand nets, beakers and siphoning tubes for different batches of larvae and ideally use separate tools for each tank. Disinfect the tools periodically and at least once a day.

4. Avoid stocking broodstock and larvae of different species in the same premises. If unavoidable, keep them separated as far as possible.
5. Disinfect the entire hatchery premises between seasons.
6. Clean and disinfect the tanks with bleach between batches and allow them to dry completely.
7. Use pathogen free live feed for the larvae. The live feed sample may be tested periodically to know the presence of pathogens.
8. Aeration to the tanks should be stopped briefly in the morning allowing debris and dead larvae to settle down. Siphon out the tank bottom to remove debris and dead larvae. Count the dead larvae as this gives an indication of the problem if there is any.
9. Check water quality parameters like dissolve oxygen, ammonia, nitrate, nitrite, temperature etc. daily. Maintain optimum water quality.
10. Excess feeding of the larvae should be avoided as it will deteriorate the water quality resulting in reduced survival.
11. If more dead larvae are found than the usual numbers, send some live larvae to the laboratory to isolate and identify the pathogens, if any, present. Tanks suspected of having an infection should be treated with disinfectants and drained immediately to prevent the spread of infection. All tools used for the infected tank, including the tank should be thoroughly scrubbed, disinfected and allowed to dry before using them again.
12. If case of high mortality, the cause of the mortality should be identified at the earliest. Larval and water samples should be sent to laboratory for testing. Further the source of the infection needs to be ascertained to prevent further infections. For this all input materials like feed and water should be tested.
13. Use of probiotics in larval rearing water is reported to be beneficial and is said to increase survivability and reduce mortality. Hence suitable probiotics may be used at the recommended dose.
14. The total bacterial load and vibrio load in the inlet water should be monitored regularly. If any increase in the load is observed, suitable measures should be taken to disinfect the water.
15. Higher stocking densities of larvae lead to stress and bacterial infections. Hence optimal stocking density should be maintained.
16. All dead fish and larvae needs to be disinfected before disposal.
17. Use aseptic techniques while collecting biopsy, hormone injection, implantation in broodfish to avoid any accidental introduction of pathogens. Disinfect the catheters, needles etc. used in sampling and injecting before each use.
18. Mullets are very sensitive to handling. Hence handle broodfish carefully to avoid stress and injury. Use anaesthetics like, phenoxy ethanol, clove oil, MS-222 to anaesthetize the fish before handing and injecting.

19. Periodically examine the brooders for the presence of any ectoparasites. If any parasites are observed treat the fish with appropriate chemicals like formalin, dichlorvas to remove the parasites.
20. Vaccinate the brooders for diseases which are endemic, if vaccines are available.
21. Observe the fish regularly for symptoms of stress/disease.
22. Educate/train all hatchery personnel on the importance of biosecurity and the do's and don'ts of the hatchery.
23. Record keeping is very much essential in hatchery operations. All activities of the hatchery should be recorded regularly.

The following are the important diseases reported in brackishwater finfish.

Bacterial diseases

Aeromonad septicemia

Aeromonad septicaemias are caused by *Aeromonas hydrophila* and to certain extend by *A. caviae*. The bacteria are ubiquitous and are commonly found in soil and water samples. It is also found in the gut and tissues of healthy freshwater and marine fishes. *A. hydrophila* are Gram negative, motile rods measuring 0.3-1.0 x 1.0-3.5 µm in size. The bacteria are not fastidious and can be easily isolated in nutrient agar. The colonies appear within 24 h and are white, circular and convex. Selective media such as Rimler-Shotts agar containing novobiocin and aeromonas isolation media containing ampicillin can be used to isolate the bacteria from samples containing other bacteria. The disease occurs when the fishes are stressed due to over-crowding, poor water quality, handling, high water temperature etc. Affected fish exhibit darkening of the skin, haemorrhages on the skin and the base of fins. Shallow ulcers may develop at haemorrhagic sites. Congestion and haemorrhages can also be observed in the internal organs. The affected fishes may be treated with antibiotics like oxytetracyline and sulphonamides. Improving the water quality with frequent water exchange and removal of dead and moribund fish will reduce mortality.

Vibriosis

Vibriosis is caused by *Vibrio anguillarum*, *V. alginolyticus* and *V. parahemolyticus*. Members of the family vibrionaceae are Gram negative, straight or curved rods. They are commonly found in marine and brackishwater environment. The causative agents can be isolated in media containing seawater salts and common nutrient media containing 1-2% sodium chloride. Thiosulphate citrate bile salt sucrose (TCBS) agar is a selective medium for isolation of pathogenic vibrio species. Colonies appear smooth, convex and white and develop within 48 hours. Vibrio species can be differentiated form Aeromonas by their sensitivity to vibriostat 0/129. The bacteria are present in the alimentary tract of normal healthy fishes. The incubation period varies with temperature and virulence of the strain.

The disease occurs in late summer when water temperature is high. Mortality may reach 50% in young fish. The affected fish are anorectic with darkening of the skin. In acute infections deep necrotic skin ulcerations with blood coloured exudate is observed. Spleenomegaly is a common feature with petechial haemorrhage on most of the internal organs. In chronic cases skin ulcers may become granulomatous. Gills become pale and corneal opacity is frequent.

Although commercial vaccines are available in developed countries, they are not available in India. Antibiotic therapy with oxytetracycline and sulphonamides is the practical method of reducing mortality during outbreaks.

Viral diseases

Viral nervous necrosis

Viral nervous necrosis (VNN) is one of the important diseases of brackishwater fishes affecting a wide range of fishes. Although mortality due to VNN is not high in mullets and milkfish, it can cause considerable loss. The causative agent of the disease, viral nervous necrosis virus (VNNV), a betanodavirus has four genotypes viz, barfin flounder nervous necrosis virus (BFNNV), redspotted grouper necrosis virus (RGNNV), striped jack nervous necrosis virus (SJNNV) and tiger puffer necrosis virus (TPNNV). The disease affects early larval and juvenile stages only. The virus is transmitted both horizontally and vertically. The virus also produces persistent infection especially in the adults resulting in asymptomatic carriers which act as a source of infection to larval and juvenile stages. Vaccination of juveniles and young adults appear promising in protecting the fish. Vaccination of brooders provides protection to larval stages through maternal transfer of immunity.

Red Sea bream iridoviral disease

Red sea bream iridoviral disease caused by red sea bream iridovirus (RSIV) has been reported in more than 30 cultured species including grey mullets. All age groups are susceptible to the virus although juveniles are more susceptible than adults. The virus affects spleen, kidney, heart, intestine and gills. The transmission of the virus is through horizontal routes. The disease is more prevalent in summer when water temperature is above 25°C. The control measures include introducing disease free fish, implementing strict biosecurity measures, and maintaining good water quality, reducing activities that induce stress, such as overcrowding and overfeeding.

Lymphocystis virus disease

Lymhocystis virus disease (LCD) is caused by Lymphocystis virus disease virus (LCDV) belonging to the genus Lymphocystivirus of the family Iridoviridae. The disease produces nodular skin lesions in a variety of fresh, brackish and marine water fishes. The nodules appear small, cream-coloured on the skin and fins externally and on the mesentries and peritoneum internally. The disease is transmitted horizontally when the lymphocysts break, through the skin abrasion caused by handling or parasites. The disease is not lethal and do not cause mortalities unless vital organs are affected. However it results in economic loss due to the poor marketability of the affected fish. The disease is diagnosed by the presence of wart like growth on the skin, gills and fin. No specific treatment is available. However the virus is sensitive to potassium permanganate, formalin and sodium hypochlorite. The warts slough off or regress if the fish are maintained well with good water quality and free of stressors like parasites. Affected fish must be isolated to prevent spread of infection.

Parasitic diseases

Argulosis

Argulosis is caused by a large ectoparasite, Argulus, commonly called as fish lice. It is the most important branchiurans parasite belonging to the family Argulidae. These parasites are dorsoventrally flattended measuring up to 1 cm in length. They are commonly found in the skin and fins of freshwater fishes and to a lesser extent in brackish water fishes. The trauma induced by the parasite due to the attachment and feeding method causes haemorrhagic ulcers and leads to secondary bacterial infection. Affected fish show lethargy, irritation and loss of appetite. It is practically difficult to eradicate argulus in culture waters as the adults and larval stages are active swimmers. Infested fish can be treated with formalin or organophosphorus insecticides. Drying the ponds and tanks between cycles will reduce argulus infestation.

Marine Ich

Marine Ich, caused by *Cryptocaryon irritans* is one of the common salt-water parasitic ciliate infestations. Some of the common signs of marine Ich are rubbing on the pond side or bottom, increased mucus secretion, breathing problems, loss of appetite, abnormal swimming behaviour, frayed fins, cloudy eyes, and white spots especially on the dorsal side. The incidence is high with poor water quality and over-crowding. The parasite infests almost all marine and brackishwater teleosts. Fishes should be quarantined and only those free of any parasites have to be taken to the hatchery. Marine Ich can be diagnosed by microscopic examination of skin and gill scrapings. *C. irritans* can be observed as 0.3-0.5 mm structures with multi-lobed nucleus. The parasite can be kept away by maintaining good water quality. Infected fish can be treated with formalin @ 100 ppm for 1 hr for 3 days or copper sulphate @ 0.5 ppm for 7 days or by immersing the fish in freshwater for one hour daily for three days.

Amyloodiniosis

Amyloodiniosis caused by *Amyloodinium ocellatum,* a dinoflagellate is one of the most frequently encountered pathogens affecting tropical marine ornamental fishes. Amyloodiniosis is also called as 'marine velvet'. The symptoms of amyloodiniosis are difficulty in breathing, sluggishness, pale gills, excess mucus secretion, rubbing its surface against objects in the aquarium and anaemia. The parasite initially infects the gill and subsequently spreads throughout the body giving a velvety appearance and thus the name marine velvet. The parasite may invade the tissues also. Affected fish appear dark in colour and emaciated. Amyloodiniosis can be easily diagnosed by microscopic examination of gill and skin scrapings. The condition can be treated with copper sulphate @ 0.5 ppm for 4-5 days or bath treatment with formalin @ 200 ppm for 1 hour. Good water quality is also advocated.

Monogenean infestation

Monogenans are ectoparasites that infest skin, gill and fins and are commonly known as gill or skin flukes. The common monogenans encountered in brackishwater fishes are Dactylogyrus, Gyrodactylus, Diplectanum and Benedenia. During heavy infections these parasites can cause high mortality in fry and fingerlings. High stocking density combined with poor water quality will result in high incidence of monogenean infestation.

Clinical signs include lethargy, high mucus production, rubbing of body against substrate, abnormal swimming behaviour and anorexia. Diagnosis is simple by observing the parasites from gill and skin scarping under microscope. Treatment includes formalin dip @ 100 ppm or dichlorvos @ 1 ppm for 1 hour.

Copepod infestation

Copepods are crustacean parasites having free living and parasitic stages. The important copepods infecting cultured brackishwater fishes are caligus spp (sea lice), Ergasilus Spp. (gill maggots) and Lernaea spp. (Anchor worm). Parasites are introduced into the culture system through water, live feed, wild fish and contaminated tools and equipment. Poor water quality and overcrowding leads to heavy infestation with copepods. Caligus can cause serious damage if present in large numbers. The damage is caused by pre-adult and adult stages which abrade the skin surface and feed on cutaneous and subcutaneous tissues. The parasite is introduced into farmed stock though introduction of wild fish. Heavy infestation with copepods results in mechanical damage, impaired respiration, petechiae, anaemia and emaciation. These parasites also act as act as mechanical vector for other bacterial and viral pathogens. Diagnosis can be done by simple microscopic examination of the gills and skin. Adult anchor worms are visible to the naked eye. Copepod infestation can be controlled using fresh water bath for 15 min. or by using hydrogen peroxide @ 1000 ppm or formalin @ 100-200 ppm for 60 minutes. Complete draining, disinfecting and drying of the tank periodically help to break the life cycle of the parasite.

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GENETIC FACTORS IN BROODSTOCK MANAGEMENT IN FINFISH HATCHERY

Rekha M.U., Krishna Sukumaran and M. Makesh

Introduction

With global population expansion, the demand for high-quality protein, especially from aquatic sources, is rising dramatically. Increased aquaculture production is clearly needed to meet this demand in the third millennium, because capture fisheries are at capacity or showing precipitous declines due to overfishing, habitat destruction and pollution. Further increases in capture fisheries are not anticipated under the current global conditions. In order to meet the requirement, the aquaculture production has to be increased. Increased aquaculture production mainly depends on the availability of quality fish seeds. The finfish hatcheries produce larval and juvenile fish for transfer to aquaculture facilities where they are grown to reach harvest size. Hatchery production confers three main benefits such as out of season production, platform to carry out genetic improvement programmes and reduced dependence on wild-caught juveniles. The production capacity of the hatchery depends on the number and efficiency of the broodstock maintained. The characters of the broodstock like growth rate, feed conversion efficiency are transmitted to their progeny and we expect the progeny to be superior to their parents. To achieve that, efficient and sustainable genetic management programmes should be followed by the hatcheries.

Quantitative genetic traits

Usually the finfish hatcheries aim at improving the quantitative traits such as growth rate, feed conversion, oxygen tolerance, percentage of body fat, meat production, disease resistance, and stress resistance. These traits are said to be quantitative because they typically vary among individuals within a population. They have a range of expression and are controlled by minor genes. These traits are measured using a continuous distribution system and statistics. Such traits are described and reported around their central tendencies, such as average (mean), variance, standard deviation and range. These traits are also influenced by the environment. Gene expression levels, the environment, and the interaction of the two can play a significant role in the variation of quantitative traits.

Genetics of small population

Hatcheries usually rear their own broodstock and do not recruit from natural water bodies or exchange breeders between farms. Each hatchery therefore can be considered as an isolated, self-sustaining and genetically closed unit (Eknath and Doyle, 1990). It is now established that in genetically closed hatchery systems, potential selective pressures exerted on finite and often small culture populations by various farm management practices such as selection of founder stock, number of breeders maintained, method of replenishing broodstock, stocking density, feeding regime etc. can result in indirect or negative selection, inbreeding and genetic drift (Doyle, 1983). Retarded growth, reduction in reproductive performance, morphological deformities, increased incidence of disease and mortality of hatchery produced seeds of carps have been reported.

There has been an unconscious negative selection in some hatcheries as the relatively bigger (hence fast growing) individuals from the grow-out ponds are sold and the remaining smaller (hence the slow growing) fish are used for broodstock replacement. This practice is also followed by nurseries in selling fast-growing; hence the good fingerlings and keeping the smaller unsold fingerlings to raise as the broodstock. This will lead to negative selection and then to poor growth and survival.

Inbreeding depression

There is every possibility of inbreeding in hatcheries as the male and female are chosen from a finite (small) population for mating, with a greater chance of crossing sib (brother-sister) or closely related fish. Moav and Wohlfarth (1976) stated that a single full sib mating of a particular fish might result in 10-20% depression in growth and considerable proportion of individuals might show physiological abnormalities. Because of generations of inbreeding and accumulation of unfavourable alleles from closed mating, genetic deterioration of existing stock might make them less suitable for culture. An inbred or homozygous population normally loses its general vigour. This result from lack of knowledge of broodstock management practices, especially about the need for recruitment of new breeders in to the stock at regular intervals, maintenance of proper stocking density of the brood fish of desirable size, injecting adequate hypophysation dosage, mating unrelated male and female breeders, basic disease control, water quality maintenance, and record keeping of broodstock and spawning. Inbreeding is not harmful always. Linebreeding is the form of inbreeding that is used to increase an outstanding animal's contribution to a population.

Genetic drift

Genetic drift is the random changes in the gene frequency that occur because of sampling error. Sampling error can be natural or manmade. Natural sampling errors are those that occur when earthquakes, floods, landslides, or other natural disasters subdivide a population and isolate small groups of organisms. Manmade sampling errors are inaccurate collections, sampling only fish that possess a certain phenotype or that spawn on a day, etc. When culturing a fish, the important change occurs in gene frequency as a result of genetic drift occurs during the creation of the next generation or during the acquisition of the population. This is when the genes are transferred from parents to offspring or when they are transferred from one hatchery to another.

The loss of alleles via genetic drift has two effects, first it increases homozygosity; consequently, it has an effect similar to that seen for inbreeding. The simultaneous effect of increase in inbreeding and loss of alleles via genetic drift as result of a decrease in effective breeding number (Ne) can cause severe genetic problems. Secondly, the loss of alleles reduces the genetic variance. Genetic material is the raw material up on which the selection works. If there is no genetic variance, there will be no heritable differences, which mean the selection cannot improve a phenotype. Equally important, if a population is being cultured for stocking lakes and rivers, the loss of genetic variance may doom the project to failure. Natural populations need broad gene pools i.e., they need as much genetic variance as possible. Populations with narrow genetic bases are likely to survive in the long term. Genetic drift has been shown to have damaged the gene pool of several hatchery populations

Measures to preserve and enhance genetic variability

a. Maintaining the effective population size (Ne)

Managing effective population size (Ne) is the most important aspect in fish husbandry. Genetic diversity of the broodstock is a major factor for the fitness of seed (Primack, 1993). Genetic variability decreases rapidly if the Ne of the brood stock is reduced. The effective population size is the number of individuals that would give rise to the rate of inbreeding appropriate to the conditions under consideration, if they bred in the manner of the idealised population. The genetic stability of a closed hatchery population depends on effective breeding size or population size (Ne). Both the inbreeding and genetic drift are inversely related to Ne.

The rate of inbreeding can be given by the formula

$$F = \frac{1}{2N_e}$$

Where, F is the rate of inbreeding, Ne is the effective population size

The relationship between Ne and genetic drift is

$$\sigma^2_{\Delta q} = \frac{pq}{2N_e}$$

Where,$\sigma^2 \Delta q$ is the variance in the change of gene frequency, and p and q are the frequencies of alleles p and q for a given gene.

The variance of the change in the gene frequency is the way genetic drift measured. There is no universal Ne that can be used to manage every population. It must be customized for every population. When managing a population's Ne, the major goal is to maintain Ne at a constant level for every generation. If Ne drops below the desired value for a single generation, the genetic goals cannot be achieved. Maintaining Ne at the desired level generation after generation may be the most difficult aspect of broodstock management, because the Ne can decline for a variety of reasons. Sudden and drastic decreases in Ne are called bottlenecks, and they can cause permanent and irreversible genetic damage. The ultimate effect of small Ne is the loss of alleles via genetic drift. Rare alleles will be lost more easily, but common alleles can also be lost. The loss of genetic variance can produce irreversible damage to the population's gene pool. Parameters of genetic variability such as allele frequency, average number of alleles per locus and average heterozygosity reveal that genetic variation decreases in the founder stock which represents the first generation of artificially propagated captive broodstock collected from wild population. Use of large number of brooder fish is recommended for minimizing genetic erosion.

b. Minimizing negative selection

A hatchery should have short term and long term plans to avoid the risk of negative selection. The following practices are recommended to minimize indirect or negative selection in hatcheries

- ❖ The base population should be collected from natural waters or from a known source.
- ❖ Record of locations of collection, date of collection/transfer, species, size and weight of the stock, number of individuals at the time of stocking in nursery/rearing ponds, etc. should be kept.
- ❖ The fast growing and best individuals from nursery/ grow out ponds should be selected for raising as broodstock and few individuals from as many stocks as possible for each species should be selected.
- ❖ The brood fish should be marked or tagged for record keeping.
- ❖ Hatchery produced seed of different selected stocks should be stocked separately or in a pool. All necessary records, like number of breeders used in each slot with their tag numbers, date of spawning, date of stocking and number individuals stocked in each nursery/ rearing pond should be maintained.

c. Avoiding stock deterioration due to inbreeding

Stock deterioration due to inbreeding can be avoided by

- ➢ Keeping an adequate number of brood fish in order to select the best performers in terms of size, maturation and breeding efficiency.
- ➢ Maintaining pedigree records to reduce or avoid the chance of mating between closely related breeders.
- ➢ Exchanging broodstock among the hatcheries to minimize inbreeding
- ➢ Adopting a well-planned selective breeding and line crossing program to improve desirable traits in the founder stocks.

DNA- marker assisted broodstock management

Because genetic factors determine the fitness and adaptability of the organisms, preservation of genetic capital of broodstock is important in the production of high quality seed for aquaculture and sea ranching. Even if founder specimens of brood stock are genetically intact, lack of a proper management strategy can lead to inbreeding and rapid decline in diversity at gene level. Deletion of wild alleles and erosion of genetic variability are known to adversely affect inherent strength and fitness of the stock. Seeds originating from genetically degraded broodstock have poor chances of survival in the wild. They have a reduced ability to respond to changing conditions of the environment (Waples et al., 1990).

Further, those that survive to maturity are likely to contaminate the gene pool of the natural populations through reproductive interaction, resulting in propagation of genotypes that are not as much adaptable as those of the wild populations. Production of seed fish for stock enhancement programs should be based on well-organised broodstock management strategies. Unknown and known genetic changes and possible loss of genetic variation in broodstock and progeny should be monitored. DNA markers are good monitoring tool for broodstock management. The information generated by these markers can be utilized in maintaining genetic variability and effective population size in broodstock and their progeny (Perez- Enriquez *et al.,* 2001)

Conclusion

The inbreeding and genetic drift are inevitable in small hatchery populations and together result in loss of heterozygosity which leads to loss or fixation of alleles and finally leaves the population homozygous for a particular allele. We can get rid of these unwanted effects by maintaining effective population size, avoiding mating of closely related individuals and by proper record keeping.

HORMONAL CONTROL OF REPRODUCTION IN FISH

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Introduction

Reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms into action. The final event of the reproductive cycle, the release of eggs and sperm resulting in spawning, can be controlled by either placing the fish in an appropriate environment or by changing the fish's internal regulating factors with injected hormones or other substances. The internal mechanism that controls the process of reproduction in fish is the brain-hypothalamus-pitutary-gonad axis. The external environmental factors that control reproduction, however, vary considerably among species. For this reason, more is known about the internal regulatory mechanism of fish reproduction than the specific environmental requirements for spawning each species.

Environmental factors

Environmental factors that regulate maturation and spawning in fishes are: photoperiod, water temperature, water quality, water current, tides and cycles of moon, weather cycles, nutrition, diseases and parasites.

Brain–Hypophyseal–Gonadal Axis

Environmental signals, in conjunction with social cues, are conveyed by sensory signals to various brain centers culminating in the hypothalamus. In the absence of hypophyseal portal system, hypothalamic nerve fibers branch throughout the adenohypophysis to regulate the synthesis and release of the gonadotropic hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Dopamine secreted by the hypothalamus may inhibit LH release. FSH and LH, in turn, bind to their respective receptors in the gonads (FSH-R and LH-R, respectively). The gonads respond by secretion of sex steroid hormones; estradiol 17 β (E2) in females that promotes oogonial proliferation and vitellogenesis, and progestogens such as 17 α, 20 β dihydroxy-4-pregnen-3-one (DHP) that promotes initiation of germ cell meiosis and follicular maturation and ovulation (Figure 1(a)). In males, these are androgens, mainly 11 ketotestosterone (11-KT), that regulate spermatogenesis and spermiogenesis, as well as DHP that initiates the meiotic division of spermatogonia and controls the spermatozoa maturation and spermiation. DHP in its free or conjugated forms serve as pheromones as well. Generally, fertilization occurs in the water, either in specialized nests guarded by one or both parents or in the open water.

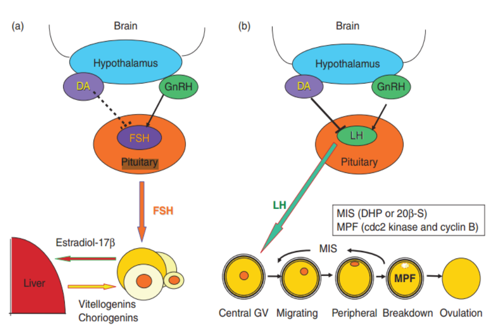

Figure 1 (a) An overview of the endocrine chain, brain–pituitary–gonadal axis (BPG axis) in model female fish during the vitellogenic phase. (b) An overview of the BPG axis during final oocyte maturation and ovulation.

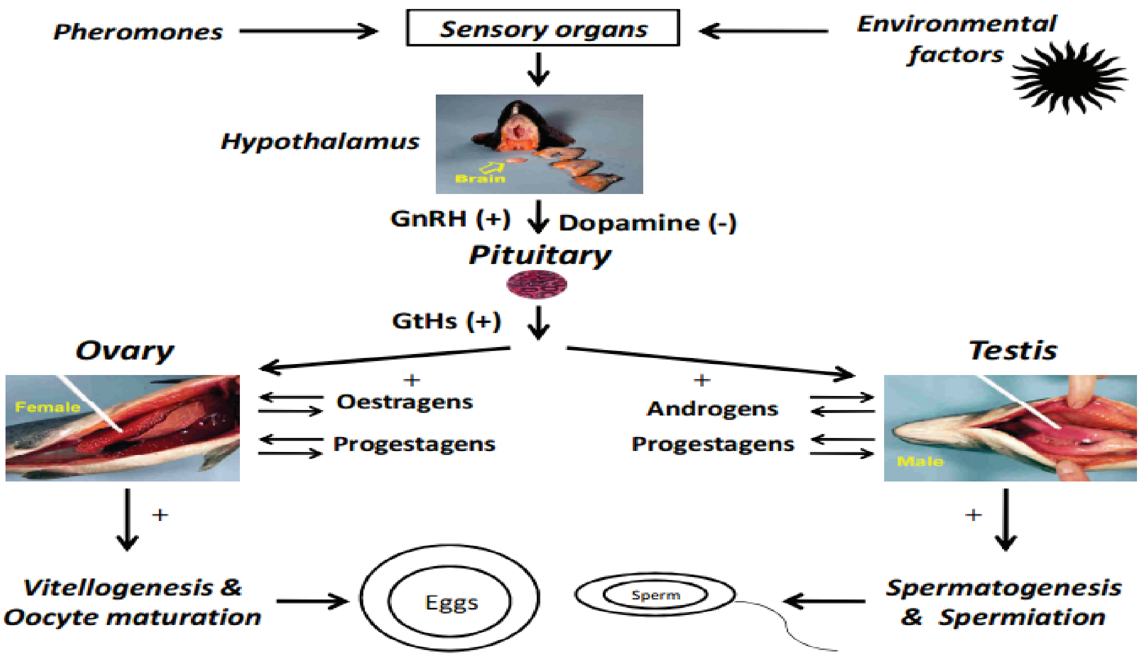

Figure1. Schematic representation of the reproductive axis in fish, its major components and phases, and its environmental and endocrine control.

Endocrinology of fish reproduction

Gametogenesis (spermatogenesis and vitellogenesis) and final maturation (spermiation and OM) are regulated by a cascade of hormones along the brain–pituitary–gonad (BPG) axis. In this axis, the secretion of the pituitary gonadotropins FSH and LH is controlled by the brain via the stimulatory action of the GnRHs, which are the primary neuropeptides regulating reproduction, acting as integrators of external information (example, environment, temperature, water fall and social interactions). Dopamine (DA) in some fishes exerts a negative effect on the functions of GnRH on the pituitary gonadotrophs. The FSH and LH are released into the bloodstream to act on the gonad, where they stimulate the synthesis of the sex steroid hormones (androgens, estrogens and progestogens), which are the ultimate effectors of gonadal development. Testicular spermatogenesis, as well as spermiation, is regulated by pituitary FSH and LH secretion through the action of the sex steroid hormones, as well as other growth factors. Before the onset of spermatogenesis, spermatogonial stem cell renewal seems to be regulated by E2 acting on Sertoli cells. The androgen 11-keto testosterone (11KT) is the major regulator of spermatogenesis, while the maturation inducing steroid (MIS) regulates sperm capaciation and spermiation. Both steroids are synthesized by the somatic Leydig cells of the testes, after GtH stimulation. The LH is mainly involved in the stimulation of androgen production in Leydig cells, whereas FSH seems to exert more complex functions in the male testes, stimulating androgen production from the Leydig cells, as well, but also regulating Sertoli cell activity during spermatogenesis. The onset of spermatogenesis is a process controlled by the secretion of pituitary GtHs (mainly FSH). The FSH acts on Sertoli cells and stimulates 11KT biosynthesis, which in turn regulates the full process of spermatogenesis, mediated also by growth factors (example, insulin-like growth factor I, IGF-I or activin B) secreted by the Sertoli cells. In males, FSH levels are high at early spermatogenesis; on the other hand, LH is low during early spermatogenesis. In males, androgen production (T and 11KT) remains high through the entire spawning period, even while MIS levels are high, since spermatogenesis, spermiogenesis and spermiation occur concurrently. In females, a predominant role has been suggested for FSH during vitellogenesis in fishes with synchronous ovarian development. On the other hand, in fish with asynchronous ovarian development the role of FSH in vitellogenesis is less clear and a possible function has been ascribed also to LH. The control of reproduction with the use LH has been worked in bakishwater fishes.

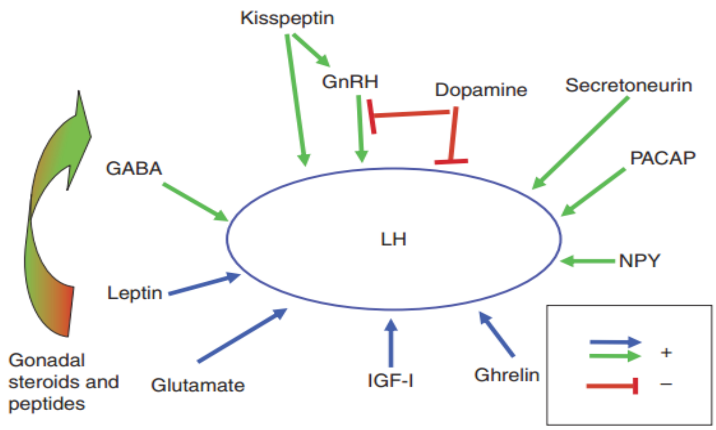

Figure 5 Regulation of LH secretion. Schematic presentation of brain and gonadal regulation of LH secretion. Hormones related to metabolism and growth are indicated by blue arrows.

Figure 3: Regulation of LH secretion. Schematic presentation of brain and gonadal regulation of LH secretion

At the conclusion of vitellogenesis, OM is triggered by the action of LH on the follicle cells, which synthesize and secrete the maturation inducing hormone (MIH) or maturation inducing steroid (MIS). In some other marine species, a derivative of 17,20bP the 17a, 20b, 21- trihydroxy-4-pregnen-3-one (20bS) has been described to act as MIS. Both 17,20bP and 20bS are acting as MIH in European sea bass, striped bass and red seabream. The MIS binds to specific receptors on the oocyte plasma membrane and the signal received in the oocyte surface is transduced to the cytoplasm to finally result in the formation and activation of the maturation-promoting factor (MPF), which is responsible for the resumption of meiosis and completion of oocyte maturation

Reproductive dysfunction in cultured

Reproduction is regulated by the brain via the release of GnRH from the hypothalamus and it stimulates the release of gonadotropin (GtH) from the pituitary. Dopamine provides a negative control of pituitary GtH release, mostly freshwater species. Fish possess two or three different variants of GnRH, and 14 variants have been so far identified from various vertebrates. Pituitary control of reproduction is via a dual GtH system, with follicle stimulating hormone (FSH or GtH I) regulating vitellogenesis and spermatogenesis, and luteinizing hormone (LH or GtH II) regulating FOM and spermiation. In female, most cultured species exhibit some degree of reproductive dysfunction when reared in captivity. Problems are more widespread in female broodstock and can vary from inconsistent spawning only, to the complete failure of oogenesis. The failure of captive fish to undergo FOM in captivity in some fish was found to be the absence of LH release during the spawning season. Fish that exhibit this type of dysfunction undergo normal vitellogenesis, but with the onset of the spawning season the developing oocytes fail to initiate FOM; instead they undergo atresia.

Treatment of such broodstock with exogenous GtH or GnRHa at the completion of vitellogenesis stimulates gonadal steroidogenesis FOM and ovulation. The final and most severe form of reproductive dysfunction of captive female broodstock is the failure to undergo vitellogenesis. During vitellogenesis, FSH or LH stimulate the production of testosterone (T) by the theca cells and its aromatization to 17β -estradiol (E2) in the granulose. In response to stimulation by E2 the liver produces vitellogenin, which is sequestered by the oocytes in a receptor-mediated process enhanced by FSH. At the completion of vitellogenesis a surge in plasma LH stimulates a drop in plasma E2, a transient increase in plasma T during GV migration, and a dramatic elevation in the plasma levels of the maturation inducing steroid (MIS), which acts at the level of the oocyte membrane to induce FOM. In male gonadotropins regulate spermatogenesis via the production of androgens by the testes, mainly 11- ketotestosterone, since T is the precursor of 11-KT, the levels of the two androgens covary during most of the reproductive season. Plasma 11-KT levels peak during spermiogenesis and decline just prior to, or during the spermiation period.

Hormones for induced spawning

Hormone-induced spawning techniques influence this sequential mechanism at several levels, by either promoting or inhibiting the process. The primary substances used for hormone-induced spawning have been: pituitary extracts and purified gonadotropins to stimulate the ovaries and testes; LHRH analogs (LHRHa) alone or in combination with dopamine blockers which enhance the potency of LHRHa to stimulate the pituitary; or steroids to stimulate the gametes directly. The appropriate hormone preparation should be selected on the basis of the species to be spawned and the availability of the hormones. Many variables impact the ability of injected hormones to induce spawning, including: 1) condition of the fish; 2) stage of sexual maturity; 3) size of the fish; 4) previous spawning history, 5) water temperature; and 6) season of the year. Pituitary extract The pituitary gland produces and stores gonadotropin hormones (GtH), which play a decisive role in ovulation and spermiation. Injected pituitary material bypasses the brain-pituitary link, acting directly on the ovaries and testes, providing the surge in blood GtH levels that normally precedes spawning just prior to spawning. This is a problem when adult fish are scarce. Fresh pituitary glands should be used immediately or preserved by either freezing or acetone-drying. Glands can simply be placed in a sterile vial or plastic bag and stored in a freezer until needed. To acetone-dry, the glands are immediately placed in a vial with acetone. After collecting the required number, the acetone in which the glands were placed is drained off and replaced with fresh acetone. The acetone is again changed 8 to 12 hours later. After 24 hours in acetone, the glands are air dried on a paper towel. The dried pituitaries are then stored in a sealed.

Commercial pituitary extracts: Common carp pituitary or salmon pituitary extracts, available commercially, are widely used for induced spawning. These are crude acetone-dried powdered whole pituitaries. As with fresh pituitaries, these preparations also contain the pituitary tissue and hormones unrelated to reproduction, in addition to GtH. In general, the closer the donor species is related to the recipient fish, the greater the chance of successful induced spawning. Therefore, carp, goldfish, Chinese carps, catfish, etc., are more likely to spawn successfully when injected with pituitary extracts from carp. Salmon, trout, etc., are more likely to spawn successfully when salmon pituitary is used. However, both are effective on a wide variety of fish species. There is always uncertainty about the hormone potency of pituitary material. Hormone content necessary for spawning is greatest in sexually mature fish just prior to spawning and lowest in immature fish and mature fish after spawning. The potency of pituitary material can also be destroyed by improper collection, processing, or storage.

Purified gonadotropin: To better quantify the hormone injected, purified gonadotropin hormones are frequently used. Human Chorionic Gonadotropin (HCG) is the most common purified gonadotropin hormone used for induced spawning. In fish, the injected gonadotropin mimics the natural GtH produced by the fish's pituitary. Just as is the case with pituitary extracts, purified hormones such as HCG bypass the brain-pituitary link, acting directly on the ovaries and testes. HCG has been used to spawn fish such as striped bass, white bass, red drum, catfish, and mullet. HCG + pituitary extract HCG, however, is not effective on all species. HCG has been used in combination with common carp pituitary extract; for some species, the combination has shown to have improved potency than either preparation used alone. The two hormones can be prepared and injected separately, or the HCG solution can be used when mixing the pituitary extract.

Luteinizing hormone-releasing hormones: Injections of mammalian Luteinizing Hormone-Releasing Hormone (LHRH) have been used experimentally to mimic the fish's GnRH. However, a comparatively large dose and frequent injections were required. Recently, synthetic LHRH analogs, referred to as LHRHa or GnRHa, have been manufactured. These hormones last longer in the fish's system and have potent stimulator effects on ovulation and spermiation in fishes. Therefore, only one or two small doses are needed to induce spawning. LHRHa stimulates the fish's own pituitary to produce and release the GtH necessary for spawning. LHRHa has been used to induce ovulation in a wide range of fishes. One of the synthetic analogs that has been used successfully is Des-GLY10 , [DAla6]-LH-RH Ethylamide.

LHRHa + dopamine blockers: Although LHRHa has not been shown to be species specific, some fish do not respond to injections of LHRHa alone (e.g., goldfish, redtailed black shark, rainbow shark). Dopamine inhibits the release of hormones from the pituitary, effectively blocking the pituitary's positive response to injected LHRHa. There is a family of drugs that act as dopamine blockers, either by preventing the release or by inhibiting the binding of dopamine. Experimental results indicate that the use of dopamine blockers prevents this negative feedback, enhancing the effectiveness of LHRHa for these species. Because of the tremendous variety of aquarium species and their individual spawning requirements, as compared to food and sport fish, development of hatchery spawning technology has been more difficult. Many ornamental species have had to be imported from wild populations. The use of LHRHa with dopamine blockers has helped change this situation. Haloperidol {4-[4-(4-chlorophenyl)- 4-hydroxy-piperidino] -4'- fluorobutyrophenone} has been used recently as a dopamine blocker in ornamental fishes and tested experimentally for food and sport fish production.

Steroids: Several steroids (e.g., progesterone and testosterone) have been tried experimentally for inducing maturation, ovulation and spermiation in fishes. However, there appears to be little indication of widespread importance of these substances for hormone-induced spawning.

Conclusions

Reproduction in fishes is regulated by both internal mechanisms within the fish and external environmental factors. The environmental factors trigger the internal mechanisms into action. The internal mechanism that controls the process of reproduction in fish is the brain-hypothalamus-pituitarygonad chain. Hormone-induced spawning techniques influence this sequential mechanism at several levels, by either promoting or inhibiting the process. The primary substances used for hormone-induced spawning are: (1) pituitary extracts and purified gonadotropin to stimulate the ovaries and testes; or (2) LHRH analogs (LHRHa) alone or in combination with dopamine blockers which enhance the potency of LHRHa to stimulate the pituitary.

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SYNTHETIC HORMONES AND THEIR ROLE IN BRACKISHWATER FINFISH BREEDING

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Introduction

Reproduction is the biological process resulting in the production of new individual. With the introduction of several species and intensification of aquaculture, control of reproduction process of fish in captivity to produce high quality seed is a major factor determining the success of the aquaculture. Unfortunately majority of the cultured teleost species exhibit some degree of reproductive dysfunction when reared in captivity. An understanding of the reproductive process in teleost fish is important for the industry to modify the timing of puberty and maturation which could aid in steady supply of fish off-season and also aid in genetic manipulations. The fish reproductive cycle are controlled by the reproductive hormones of the brain, pituitary and gonad. The nervous and the endocrine system works together to control reproduction through the hypothalamo-pituitary-gonadal (HPG) axis. The main hormones involved are the hypothalamic decapeptide gonadotropin-releasing hormone (GnRH), gonadotropins (GTHs) from the pituitary and the sex steroids from the gonads. In addition to steroids, several factors such as stress and nutrition are involved in the control of the HPG axis to modulate the release of gonadotropin release.

Hormonal therapies

Although the growth phase of reproductive development is concluded in captivity in most fishes-the major exemption being the freshwater eel (Anguilla spp.), oocyte maturation (OM) and ovulation in females and spermiation in males may require exogenous hormonal therapies. 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. The findings that heterologous pituitary homogenates could induce females to ovulate lead to the development of the hypophysation technique which was used extensively in aquaculture species. The exogenous LH preparations act directly at the level of the gonads to induce FOM and ovulation. However, the technique was later replaced by methods employing either human chorionic gonadotropin (hCG) extracted from the urine of pregnant women, or piscine pituitary extracts and purified LH obtained through chromatographic separation. Of the gonadotropin preparations, hCG often in combination with GnRHa, has been tested with variable success in many commercially important fish. The major drawbacks of all GTH preparations is their high cost and the fact that fish may become refractory to similar treatment in subsequent spawning seasons.

Dopamine, a catecholamine neurotransmitters, exerts inhibitory effect on some fish. Secretion of dopamine from nerve terminals in the pituitary and its binding to D2 receptors localized on gonadotrophs results in inhibition of basal and GnRH-stimulated release of LH. Treatment with a DA antagonist causes an increase in the numbers of LH-like gonadotrophs and is directly proportional to time and the dose of the antagonist. The inhibitory effect of DA on LH secretion changes over the course of the reproductive cycle, with the maximum DA inhibition occurring during the final stages of gametogenesis. This feature is utilised in aquaculture of Cyprinidae by using dopamine antagonists in ovulation-inducing therapies, e.g., domperidon, pimozide, reserpin, metoclopramide, haloperidol, isofloxythepin .

The use of synthetic GnRH and its hyperactive agonists for maturation induction commonly employed, trigger the secretion of the fish's own GTH, thus activating its pituitary-gonad axis. GnRH being a small decapeptide apparently does not trigger an immune response and acts at a higher level in the BPG axis, thus providing a more balanced stimulation of reproductive events. GnRHa is synthesized chemically and does not carry the risk of transmitting diseases to the broodstock, a danger always associated with the use of pituitary extracts. The development and utilization of synthetic GnRHa in fish culture has greatly increased the level of sophistication and control of hatchery production and contributed substantially to the growth and diversification of the aquaculture industry. However, injection of GnRHa does not always result in 100% ovulation and often multiple injections are often necessary to induce ovulation. Thus there was a need for the development of a hormonal formulation that does not require repeated applications in the development of spawning induction therapies which lead to the development of controlled-release delivery systems for GnRHa which has proven to be an important broodstock management tool, and have contributed to the species diversification of the aquaculture industry in the last decade. The hormones implants mixed with cholesterol, ethylene-vinyl in biodegradable microspheres have been efficient in inducing maturation and spawning in many cultured fish.

Kisspeptins are involved in the onset of puberty by the activation of the HPA axis in fish. Exogenous kisspeptin injection either centrally or peripherally causes stimulation of the HPG axis in many mammalian as well as in teleosts. *Cirrhinus mrigala* was induced using synthetic Kissppetin-10 hormone alone and in combination with domperidone. Central as well as peripheral administration of KP robustly increases systemic levels of the LH and FSH in sexually immature and mature rodents

A major concern with regard to hormonal therapies is their effect on gamete quality, compared to naturally maturing or spawning broodfish. The main factors that may have significant consequences on gamete quality-mainly on eggs-and should be considered when choosing a spawning induction procedure include (a) the developmental stage of the gonads at the time the hormonal therapy is applied, (b) the type of hormonal therapy, (c) the possible stress induced by the manipulation necessary for the hormone administration and (d) in the case of artificial insemination, the latency period between hormonal stimulation and stripping for in vitro fertilization.

Commercially used synthetic compounds for induced breeding

1. *Gonadotropins:*

Three types of gonadotropins are generally used for fish breeding. Mammalian luteinizing hormone (LH); Human chorionic gonadotropin (hCG); Pregnant mare serum gonadotropin (PMSG). HCG has different brand names like Prolan, Antuitrin, Sumaach and Synahorin which contains mammalian pituitary extract also.

2. *Synthetic Gonadotropin relaeasing hormone:*

Scientists were successful in synthesizing analogues of GnRH substituting amino acids at position 6,7 or 10 to make GnRH or LHRH analogues (GnRH-a/ LHRH-a). Commercially available forms are- Ovaprim containing Salmon GnRH-a (sGnRH-a) and domperidone (Syndel Laboratory), Ovatide containing GnRH-a and pimozide (Hemmopharma), Ovapel available in pellet form containing mGnRH-a and metoclopramide (University of Gödöllo), WOVA-FH containing GnRH-a (Biostat Agrisciences, Wockhardt).

3. *Steroid Hormones:*

11- deoxycorticosterone acetate (DOCA), 17 alpha, 20 beta di-hydroxy progesterone (17α,20β-DHP), 17α Methyl Testosterone (17-MT) has been extensively used for fish breeding.

4. *Other drugs:*

Prostaglandin and Antiestrogens like Tamoxifen has been used to stimulate natural spawning and inducing ovulation in fishes in captivity.

Induced breeding of captive reared brackishwater fishes at ICAR-CIBA

Understanding of sex-steroid pathway in wild fish and manipulating it in captivity through hormonal manipulation is essential to achieve gonadal recrudescence. Carnivorous and herbivorous fishes differ significantly in reproductive physiology particularly in the area of final oocyte maturation (FOM) and the age of first maturity. Brood fishes at CIBA are ideally maintained in 100 ton RCC tanks or earthen ponds with flow through system having salinity > 29 ppt. Carnivorous finfishes like sea bass (*Lates calcarifer*), grouper (*Epinephelus tauvina*) and cobia (*Rachycentron canadum*) achieves final oocyte maturity and spawning only under hormonal manipulation (neuro-peptides and sex-steroids). Gonads of carnivore fishes have characteristic feature of spontaneous maturity based on brood nutrition with trash fishes (@ 5% body weight) and spawning takes place only after final LHRH-a (75 mg/kg) /HCG (250 IU/kg) injection when oocyte reaches 450 µm and 650 µm in sea bass and cobia, respectively. On the other hand, herbivorous fishes need sustained release of GnRH throughout its reproductive cycle to mature and spawn. Milkfish (*Chanos chanos*) and grey mullet (*Mugil cephalus*) were given intra muscular implantation of LHRH-a (50 mg/kg) and 17 α –Methyl Testosterone (50 mg/kg) at monthly interval to mature. Milkfish and mullet spontaneously spawn when oocyte diameter reaches 850 µm and 600 µm respectively after several intramuscular hormone implantations. *Mystus gulio*, a high valued fish locally known as "Nuna Tengra" was induced to attain oocyte maturation under captive condition through dietary manipulation and LHRHa hormone pellet implantation. Fish were observed to spawn after 4 – 6h of injection. Breeding trials in the gold spot mullet, *Liza parsia*, a species with high consumer demand in West Bengal and Orissa, showed that oozing males and females with developing ovary following treatment with LHRHa. Broodstock feed containing high protein and lipid along with other vital micronutrients plays a major role in achieving maturity. Proper monitoring of brood-stock health, nutrition and water quality with controlled manipulation of environment (salinity and temperature) is most important factors in marine herbivores.

Conclusion

Among the significant advancements in the field of aquaculture is the development of techniques to induce reproduction in fish. The multiplicity of neuroendocrine signalling pathways in teleosts is probably due to the gene duplication event but several evidences have suggested their unique roles and functional significance in a variety of reproductive strategies in teleosts. Numerous hormones have been used to induce reproduction of a majority of economically important fishes. These techniques have allowed farmers to profitably breed and raise species that do not naturally reproduce in captivity, and to manipulate the timing of reproduction to suit production cycles. Compared to multiple injections, sustained-release GnRHa-delivery systems reduce the necessary handling to a minimum. Although major advances have been done over the last years in our understanding of these central mechanisms, there still are a number of unresolved issues that represent true bottlenecks for the development of sustainable aquaculture**.** Further research aimed at understanding the reasons for reproductive dysfunction should contribute to future progress in the area of artificially stimulation of oocyte and maturation and ovulation of fish in captivity.

SOIL AND WATER QUALITY MANAGEMENT OF FINFISH HATCHERIES AND CULTURE PRACTICES

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Introduction

Aquaculture, as the aquatic equivalent of agriculture and terrestrial animal husbandry business.It has numerous and often poorly defined risks, both biological–physical and social–economics, and characterized by considerable variability as most biological processes, including meteorological and other abiotic uncontrolled effects, which are interrelated (Webber, 1973). The steadily growing importance of aquaculture has compelled improvements in the technologies necessary for securing the initial and basic requirements for productive aquaculture; namely the production of fish seed for stocking. A fish hatchery is a place for artificial breeding, hatching and rearing through the early life stages of animals. It consists of different units like egg collection, incubation, larval rearing for culture and nursery rearing facilities. The efficient operation of a fish hatchery depends on a number of factors such as suitable site selection, soil characteristics and water quality. The site selection should be a broad, sound and careful process. In the majority of the cases and especially on those for hatcheries, the most important aspect is water quality in relation with species, live stages and culture systems. Site selection for aquaculture facilities should be based on culture potentialities assessments of sites, according to needs on different aspects and criteria, especially (a) species to be work with, its life-cycle requirements and tolerance limits of main environmental parameters; (b) present and future working objectives that should be accomplished; (c) purposes of the facility (educational, research, production, etc.); (d) working scale (experimental, pilot, commercial, and extension-demonstration); (e) working aspects (seed production, nursery, and grow-out); (f) type of system that would be used (enclosures, fish pens, cages, ponds, and tanks); (g) system intensity (extensive, semi-intensive, intensive, and super intensive).

Important Water Quality Parameters affecting hatchery production:

Water quality determines to a great extent the success or failure of a fish cultural operation. Water is the culture environment for fish and other aquatic organisms. It is the physical support in which they carry out their life functions Water quality is one of the most critical factors besides good feed/feeding in fish production. For a successful aquaculture venture, the dynamics and management of water quality in culture media must be taken into consideration. Water quality parameters can be divided into three main categories: physical (density, temperature); chemical (pH, conductivity, nutrients) and biological (bacteria, plankton and parasites. All living organisms have tolerable limits of water quality parameters in which they perform optimally. A sharp drop or an increase within these limits has adverse effects on their body functions.

1. Water Temperature:

It is important to note that intrinsic differences exist in adaptation of fish to water temperature. For each species, there exists an upper and lower limit, as well as an optimum range for growth, which changes with development. The temperature for optimum growth of fish is called the SET, standard environmental temperature. Most warm water fishes need water temperatures ranging from 20 to 30ºC for their propagation.

Fish are stressed and disease outbreaks occur after a sudden temperature change or when temperatures are chronically near their maximum tolerance. The metabolic rate of ectothermal animals is said to double with each 10°C rise in temperature, a relationship called the Q_{10} factor. Temperature controls the solubility of gases in water, and the reaction rate of chemicals, the toxicity of ammonia, and of chemotherapeutics to fish.

Extreme cold or warm temperatures inhibit final gonad development. The optimum temperature range for development of eggs and rearing of fry is 26 to 28°C. Cobia requires 23-27°C during spawning. If the temperature is too low, hatching and development are prolonged. At higher water temperatures, embryos develop too fast and there may be a high incidence of malformed or nonviable fry.

Considerable energy is required to heat or cool water and it is usually too costly to attempt major changes in water temperature. Therefore, the temperature of the water supply should be near 80°F before it enters the hatchery. An in-line water heater can be used to ensure a minimum temperature in the inlet water. The opposite condition may be encountered late in the spawning season when surface waters become too warm for use in hatcheries.

2. Dissolved gases in water:

In addition to the atmospheric gases natural waters contain additional dissolved gases that result from erosion of rock and decomposition of organic matter. Oxygen (O_2) and carbon dioxide (CO_2) are important molecules because they are involved in photosynthesis and respiration processes. Nutrients like nitrogen are essential on biological metabolism. But when is excreted by the fish as ammonia (NH_3), in certain circumstances it can be toxic and even lethal in high doses. Besides the physical and chemical factors, the biotic component also can change the water composition. For example, algae can consume of produce oxygen and carbon dioxide, depending on light presence or absence.

a. Dissolved oxygen:

Oxygen is the first limiting factor for growth and well-being of fish. Fish require oxygen for respiration, which physiologists express as mg of oxygen consumed per kilogram of fish per hour (mg O_2 /kg/h). The respiratory rate increases with increasing temperature, activity, and following feeding, but decreases with increasing mean weight. At a given temperature, smaller fish consume more oxygen per unit of body weight than larger fish. Therefore, for the same total weight of fish in a tank, smaller fish require more oxygen than larger fish. Actively swimming fish consume more oxygen than resting fish. Oxygen consumption of fish will increase after feeding; multiple feedings per day (3 or more) will result in less variation in oxygen demand than 1 to 2 feedings per day. The oxygen consumption rate of fish of different species ranges range from 200-500 (mg O_2 /kg/h). Oxygen concentration at saturation in a given elevation varies in relationship to water temperature. Oxygen concentration should not be less than 70% of saturation.

In ponds, the major source of oxygen is from algal photosynthesis and from wind mixing the air and water. In tanks oxygen is supplied by the inflowing water, which should be near saturation for the temperature. A common generalization about oxygen requirements for aquaculture is that the minimum DO should be greater than 5 mg/L for growth of warm water fish. Thus for a circular tank, oxygen of the effluent water should be at least 5 mg/L. At temperatures optimum for growth, fish are stressed at if the available oxygen concentrations less than 5 mg/L. If the condition is chronic, fish stop feeding, growth slows down, stress-related disease begins.

b. Carbon dioxide:

The primary sources of carbon dioxide in fish ponds are derived from respiration by fish and the microscopic plants and animals that comprise the fish pond biota. Decomposition of organic matter derived from unfed feed and excreta matter is also a major source of carbon dioxide in fish ponds. The problem with the potential toxicity of carbon dioxide can be related to the daily fluctuating pattern of dissolved oxygen and carbon dioxide concentrations. Carbon dioxide concentrations are highest when dissolved oxygen concentrations are lowest. If environmental carbon dioxide concentrations are high, the fish will have difficulty reducing internal carbon dioxide concentrations, resulting in accumulation in fish blood. This accumulation inhibits the ability of haemoglobin, the oxygen carrying molecule in fish blood, to bind oxygen, and may cause the fish to feel stress similar to suffocation.

Carbon dioxide concentrations are maximum during winter and minimum during summer. Warm water temperatures increase the metabolism of all pond organisms and therefore respiration rates are high. It is also a time of year when feeding rates are high. The decomposition of wastes generated by large quantities of organic matter added to fish ponds in the summer requires large quantities of dissolved oxygen and produces large quantities of carbon dioxide. High levels of dissolved carbon dioxide interfere with respiration by eggs and fry concentrations up to at least 10 ppm seem to be well tolerated, provided that dissolved oxygen concentrations are adequate.

3. Salinity:

Salinity is the dissolved salt content of water and is often expressed as the parts of salt by weight per thousand parts of water by weight (ppt). Salinity and dissolved solids are made up mainly of carbonates, bicarbonates, chlorides, sulphates, phosphates, and possibly nitrates of calcium, magnesium, sodium, and potassium, with traces of iron, manganese and other substances. Spawning is one of the processes affected by the salinity of water. Some fish migrate from marine to freshwater environment while others do vice verso for spawning and complete their life cycle. Asian Seabass requires 28-32 ppt for reproduction. Eggs can hatch and fry will develop in waters with salinities up to at least 32 parts per thousand.

4. Turbidity:

Turbidity is the term associated with the presence of suspended solids. Analytically, turbidity refers to the penetration of light through water (the lesser the penetration, the greater the turbidity. Turbidity can be caused by many substances, including microscopic algae (phytoplankton), bacteria, dissolved organic substances that stain water, suspended clay particles, and colloidal solids. Turbidity caused by clay particles is generally undesirable because it keeps light from penetrating the water, and light is required for algal growth. At very high concentrations, clay particles can also clog fish gills or smother fish eggs. Turbidity in excess of 100,000 parts per million do not affect fish directly and most natural waters have far lower concentrations than this. In general, turbidity less than 2,000 parts per million is acceptable for fish culture.

5. Alkalinity and Hardness:

Alkalinity is the buffering (alkaline) capacity of the water. Alkalinity is a measure of the capability of water to neutralize acids. In most natural waters, the predominant bases are bicarbonate and carbonate. Alkalinity is expressed as ppm equivalent $CaCO_3$.Fish eggs and fry thrive in waters with a wide range of alkalinity, although waters of very low alkalinity (<10 ppm as $CaCO_3$) should be avoided as hatchery supplies if possible. These waters are poorly buffered and pH can fluctuate drastically with small additions of acid or base. More importantly, dissolved metals such as copper and zinc are very toxic to fry in waters of low alkalinity. Waters with high alkalinity are undesirable because of the associated excessive hardness or high concentrations of sodium salts. So water with alkalinities between 120-400 ppm is optimum.

Hardness refers to the amounts of calcium and magnesium in the water and is expressed as ppm of equivalent CaCO3. Adequate concentrations of environmental calcium are required for "hardening" of eggs and for normal bone and tissue development of fry. A minimum of 5 ppm calcium hardness is required for adequate egg hatchability and for development and vigour of sac fry. Higher calcium concentrations are desirable because calcium also protects fry from ammonia and metal toxicities. All things considered, hatchery water supplies should contain at least 20 ppm calcium hardness.

Potential Hydrogen (pH/Acidity):

Acidity refers to the ability of dissolved chemicals to "donate" hydrogen ions (H^+). The standard measure of acidity is pH, the negative logarithm of hydrogen-ion activity. The pH of most productive natural waters that are unaffected by pollution is normally in the range of 6.5 to 8.5 at sunrise, typically closer to 7 than 8. The controlling factor for pH in most aquaculture facilities is the relationship between algal photosynthesis, carbon dioxide (CO_2), and the bicarbonate ($HC0_3^-$) buffering system. At night, respiration by bacteria, plants, and animals results in oxygen consumption and carbon dioxide production, producing carbonic acid (H_2C0_3), then bicarbonate $HC0_3^-$ and H^+ions; the increase in H^+causes the pH to drop. During sunlight, respiration continues, but algae use CO_2 for photosynthesis reduction in CO_2 level consume H^+ for $HC0_3^-$ reducing the abundance of H^+ ions, and pH goes up. Fish can die from pH shock, a consequence of a sudden change in pH (1.7 pH units) that may occur when moving fish from pond to tank, or tank to pond. Toxicity of other compounds to fish, especially ammonia and chlorine, are affected by pH.

6. Presence of Metabolites:

a. Ammonia:

Of all water quality parameters, which affect fish, ammonia is the most important after oxygen. Ammonia is the principal nitrogenous waste product of fishes that represents 60% to 80% of nitrogenous excretion of fish. It is also, the main nitrogenous waste material excreted by gills beside urea and amines and an end product of the protein catabolism. In water, total ammonia consists of non-toxic (ionized ammonia) referred to as ammonium (NH_4^+) and toxic un-ionized ammonia (NH_3). The equilibrium between these two forms is pH and temperatures dependant. Toxicity from high TAN is more likely at high pH and high temperatures, conditions that occur in mid-summer in ponds with high standing crop of fish, which are also likely to have a heavy algal bloom, and mid-afternoon pH values close to 9.The NH_3 molecule is soluble in lipids. It is

300 to 400 times more toxic than NH_4^+. Un-ionized ammonia (UIA-N) can readily diffuse across the gill membranes due to its lipid solubility and lack of charge. Ammonia tends to block oxygen transfer from the gills to the blood and can cause both immediate and long term gill damage. Also it can cause impairment of cerebral energy metabolism, damage to gill, liver, kidney, spleen and thyroid tissue in fish.

b. Nitrite:

Nitrite an intermediary product of bacterial nitrification is highly toxic to fish. A more rapid growth rate of *Nitrosomonas* in the biological system can lead to accumulation of nitrite. It oxidises the haemoglobin to methemoglobin which is incapable of transport of oxygen. As the pH increases nitrite toxicity increases. Nitrite toxicity decreases slightly as the hardness and chloride content of water increases.

Management of water quality in finfish seed production:

In brood stock tank stocking density should be maintained at 1 kg/m^3 in the. The stocking density in cages can be doubled depending upon the water quality and feed management. Bloodstock maintained in captive condition should be provided with the environmental quality prevailing in the sea for maturation and spawning. Even if not all conditions, the water quality should be maintained to maximum extent to that of sea water. The source water should be passed through high pressure sand filters to remove the impurities and toxicants. The problem of algal bloom is minimal in flow through system and tanks with over cover. Tanks open to sky prone to aquatic weeds which inhibit the light penetration and restrict the fish movement and feeding. To overcome the problem of aquatic feed, removal of unfed feed and metabolites the tank should be cleaned in the early morning hours by reducing the water level to a minimum. After cleaning bottom and walls of tank replacing the water results in exchange of 70 -80% water daily. In flow through system the water quality parameters should be monitored regularly and necessary actions should be taken depending upon water quality. Dissolved oxygen in the brood pond should remain above 5 mg/L at all times for successful spawning. If a tank is stocked with fish, over several weeks of a growth cycle, the fish will grow, reducing their consumption rate (inverse OC-fish size relationship), but the density (kg/m^3) will increase. Flow to the tank will have to be increased or the population divided to handle the larger oxygen demand. Under emergency conditions, application of dissolved oxygen enhancers improves the water quality. Water exchange is the best solution to prevent low DO problem in brood stock tank. Aeration and mixing are the most effective available mechanical methods for the management of carbon dioxide and dissolved oxygen. Vigorous aeration accelerates the diffusion of carbon dioxide out of water and mixing will prevent or minimize the establishment of a carbon dioxide-rich layer of water near the pond bottom. Maintaining a moderate plankton density (Secchi disk visibility between 6-12") will maximize the biological uptake of carbon dioxide. The salinity of the brood stock and spawning tank should be same. If there is any variation of salinity from the optimum level it can be restored by mixing with brine solution or freshwater. After 2 – 3 days when fish got acclimatized to the spawning tank conditions, the salinity of the water is reduced to 24 ppt. The fishes are maintained in this condition for about a week and then the salinity is increased to 30-32 ppt by daily water exchange over a period of 10 days. Ammonia and nitrite level can be maintained below the critical limit by providing aeration, removal of debris and plankton and water exchange.

Rearing of hatchlings through the various developmental stages providing required environmental parameters and feed is the most important phase in the seed production technology. Water quality in the rearing tanks is very important for better survival and growth of the larvae. Water provided to the larval rearing tanks should be free from flagellates, ciliates and other unwanted pathogenic organisms. Water should be filtered through biological filters; pressure sand filters and UV radiation filters for get rid of pathogenic organisms. Residual chlorine should be removed if chlorine treated water is used because fish larvae is highly sensitive to chlorine. The bottom debris in larval rearing tank should be removed by siphoning with a filter net of 100-200µ upto 9 days and 200-400 µ mesh size afterwards. The salinity of the water should be maintained to a level of 25- 30 ppt and a temperature of 27-29^{o}C. The temperature can be maintained by heaters during winter season. Un-ionized ammonia is quite toxic to sac fry and early swim-up fry. Ideally, water in rearing troughs should be free of ammonia for optimal health and growth of fry, and the maximum concentration of un-ionized ammonia that should be allowed is about 0.05 ppm NH_3-N. Above this concentration, fry develop more slowly and are more susceptible to infectious diseases.

Soil and water requirements for brackish water finfish grow out culture:

Soils are a major factor in pond aquaculture and the condition of pond bottom influences water quality and production. Concentrations of nutrients and phytoplankton productivity in pond water are related to pH, and nutrient concentration in soils. Before initiating aquaculture operation, one should be well acquainted with the nature of soil as it affects the fish production.

Soil texture has direct effect on the productivity of ponds. Soils with moderately heavy texture such as sandy clay, sandy clay loam and clay loam are highly suitable for aquaculture. In general soil pH ranging between 6.5 and 7.5 are best suited for brackish water environment. Under this pH range, the availability of nitrogen, phosphorus, potassium, sulfur, calcium and magnesium concentration is maximum. Soil rich in $CaCO_3$ content promotes biological productivity as it enhances the breakdown of organic substances by bacteria creating more favourable oxygen and carbon reserves. The productive soil should have calcium carbonate more than 5%. Organic matter is an important index of soil fertility. It helps in prevention of seepage loss, increases arability of pond bottom and supplies nutrients. It reduces turbidity of pond water and act as antioxidants. Organic matter influences microbial activity and productivity of pond. Soil which has organic carbon content less than 0.5 % is low productive, 0.5-2% is medium productive and > 2% high productive. Optimum value is 1.5-2%. In sediments, when organic matter exceeds the supply of oxygen, anaerobic condition develops. This reducing condition can be measured as the redox potential and is represented as Eh. The redox potential of mud should not exceed -200 mV.

Conclusion:

Fish culture today is hardly possible without the artificial propagation of fish seeds of preferred cultivable fish species. The need for the production of quality fish seed for stocking the fish ponds and natural water bodies has indeed increased steadily. Water is the physical environment where fish develop, growth and reproduces. Successful hatchery operations can be possible by only use of good quality water and regular monitoring of water quality parameters and maintaining it by different measures such as water exchange, aeration, etc. To ensure sustainable fish production, soil and water quality are two major parameters. Daily monitoring of the pond conditions and fish behaviour along with accurate record keeping helps the farmer to recognize and prevent deleterious environmental conditions in the pond and there by maximize the production and profit.

Appendix:

Table 1: Suggested water quality parameters for water resources

Parameter	Value
pH	6.5 – 9.0
Dissolved oxygen	5 ppm – saturation
Carbon dioxide	0 – 10 ppm
Total alkalinity (as $CaCO_3$)	50 – 400 ppm
Total Ammonia Nitrogen (TAN)	0 – 0.05 ppm
Nitrate	0 – 3.0 ppm
Nitrite	0 – 0.05 ppm
Phosphate	0.01 – 3.0 ppm
Manganese	0 – 0.01 ppm
Iron	0 – 0.5 ppm
Zinc	0 – 0.05 ppm
Lead	0 ppm
Hydrogen sulphide	0 ppm

(Saraswathy et al., 2012)

Table 2: Desirable range of some of the water quality parameters in a brood stock tank

Parameter	Value
pH	7.0 – 8.2
Dissolved oxygen	More than 5 ppm
Carbon dioxide	0 – 10 ppm
Total alkalinity (as $CaCO_3$)	50 – 400 ppm
Total Ammonia Nitrogen (TAN)	Less than 0.1 ppm
Nitrite	Less than 0.01ppm
Phosphate	10-20 ppm
Suspended solids	2 - 5 ppm

(Thirunavukkarasu et al., 2013)

Table 3: Desirable range of some of the water quality parameters in a larval rearing tank

Parameter	**Value**
Temperature	28-30^{o}C.
Dissolved oxygen	More than 5 ppm
pH	7.0 – 8.2
Total Ammonia Nitrogen (TAN)	Less than 0.1 ppm
Nitrite	Less than 0.01ppm
Phosphate	10-20 ppm
Suspended solids	2 - 5 ppm

(Kailasam et al., 2013)

Table 4: Soil requirements for brackish water finfish culture

Parameter	**Value**
pH	6.5-7.5
Electrical conductivity (dS/m)	>4
Clay content (%)	18-35
Organic carbon (%)	1.5-2.0
Calcium carbonate (%)	>5
Available nitrogen (mg/100g)	50-70
Available phosphorus (mg/100g)	4-6

(Saraswathy et al., 2015)

Table 5: Optimum water quality parameters for fish culture ponds

Parameter	**Value**
pH	6.5 – 9.5
Dissolved oxygen	>5 ppm
Carbon dioxide	0 – 10 ppm
Total alkalinity (as $CaCO_3$)	50 – 400 ppm
Total Ammonia Nitrogen (TAN)	0 – 2 ppm
Un ionized ammonia (NH_3)	<0.02 ppm
Nitrate	<0-2.5 ppm
Nitrite	0 – 1 ppm
Phosphate	0.01 – 3.0 ppm
Turbidity	50 cm
Temperature	25-32^{0}C
Total suspended solids	<25 ppm

(Saraswathy et al., 2015)

LARVAL NUTRITION AND FORMULATED LARVAL FEEDS FOR FINFISH LARVAE WITH SPECIAL REFERENCE TO MILKFISH AND GREY MULLET

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Background

Although live feeds provide an excellent source of nutrition, there are several drawbacks associated with their use. Algal cultures require considerable expertise to maintain them in peak nutritional condition and facilities for their mass production can be expensive to operate. Rotifers also require considerable expenditure in time and effort to maintain, especially if they, in turn, need to be provided with live feed.

Live Artemia nauplii suffer from inconsistent supply and quality as they are obtained from cysts collected in the wild environment. The bulk of cysts come from the Great Salt Lake in Utah in the US where annual fluctuations have been shown to cause wide fluctuations in yield. As a result, price and quality can vary unpredictably. Such problems with live feeds have led to the development of diets specifically formulated for their replacement. However, the development of formulated larval diets to completely replace live feeds has been an elusive goal, despite considerable effort

Milkfish and Mullet fish larvae are very susceptible during the first stages of development and have strict requirements for biotic and abiotic conditions to survive, develop and grow properly. There are reported problems such as larval mass mortalities, incidences of deformities and variable production are still experienced. Improvements in larval nutrition are necessary to solve some of these problems to come up with a viable and dependable milkfish larviculture technology for commercial scale application.

Another aspect to take into account is the variety in ontogeny, feeding physiology and nutritional requirements among species, even within the same family. Consequently, many specific processes cannot directly be extrapolated from findings obtained in model species and require specific studies.Obviously, a good knowledge of the larval nutritional requirements throughout development would contribute to optimize diets and feeding protocols, and thereby improve larval and juvenile quality. Nevertheless, considering the vulnerability of fish larvae, it is always difficult to identify and meet nutritional requirements when several physiological and metabolic constraints are linked and each of them may prevent growth or an appropriate development. The nutrition of fish larvae also involves a consideration of the behavioural and physiological processes of feeding. These are likely to be varying in the larval stages compared to adults.

An integrated understanding of the different factors and events interacting in the food acquisition and processing is necessary for designing larval diets that meet the larval requirements for optimal ingestion, digestion and absorption of nutrients. Finally cost is also a critical factor which matters in scaling up the larval feeds for commercial use particularly in species like milkfish and mullet which are low value food fishes. Considering all these limitations and based on the analysis of the current information available in marine fish nutrition, ICAR-CIBA involved in functional hatchery feeds s to identify the most burning gaps to be addressed in future research to achieve a more efficient production of high quality fish larvae and fry.

Larval nutrient requirements

We know very little about the nutritional requirements of milkfish and mullet larvae. Both qualitatively and quantitatively they may differ from those of juveniles or adult fish, since fish undergo dramatic morphological and physiological changes, including metamorphosis, during ontogenesis. Moreover, fish larvae grow extremely rapidly, feed continuously and, therefore, the total ingestion of nutrients must be high. Fish larvae need optimal requirements of protein, lipids, carbohydrates, vitamins and minerals.

The requirement for a particular nutrient can be defined from a physiological point of view as the nutrient intake needed to fulfil a physiological role. However, the design and formulation of diets requires translation of the nutritional requirements into the nutrient content in the diet. Micronutrient requirements, but also requirements for protein / amino acids, fatty acids and so forth, are often given as dietary concentrations / fractions, and, expressed in this way requirements do not always increase under demanding conditions, such as high growth rates and metamorphosis.

Nutritional requirements are frequently defined as the 'requirement for maximal growth and / or survival' where the relation fish-diet-feeding has an important effect in the determination of the quantitative needs.

- HUFAs and phospholipids. Both DHA and EPA are essential for the normal growth and development. EFA deficiency signs include poor growth, low feed efficiency, anemia and high mortality.
- 1% n-3 HUFA in the diet could be considered a minimal value for PL.
- Dietary sources of phospholipid has been shown beneficial, possibly through enhancing the absorption of dietary cholesterol and triacylglycerols.
- The phospholipid requirement of fish larvae in general appears to be within the 1-3% range.
- Dietary protein level recommended in larval feeds varies 45-60%.
- Ascorbic acid, or vitamin C, is required in larval fish diets. Scoliosis, distorted twisted gill filaments, short operculae and snout are some of the gross signs of ascorbate deficiency.
- Vitamin A is involved in vision, growth, bone development, reproduction and normal maintenance of epithelial tissues. The studies on vitamin A in fish larvae are largely focused on the effects on skeletal development.

Different types of formulated larval feeds

Four types of "formulated diets" are generally available

Microbound diets

- Nutrients are bound within matrix (agar, gelatin, other)
- Prepared in form of slurry and then dried, ground and sieved to collect particles of defined size
- Potential problem is nutrient "leaching" into water

Microencapsulated diets

- Nutrients are enclosed within microcapsule wall or membrane
- Reduces problem of nutrient leaching
- Used to date primarily with as co-feed with microalgae

Flakes

- The formula ingredients are rolled in to flakes in the presence of steam.

Granulated feed

The formula ingredients are extrusion cooked in the presence of steam.

Liquid diets

the formula ingredients will be in suspension as a concentrated soup with preservatives

Desired characteristics of artificial diets for fish larvae

Characteristic	Explanations
Acceptability	Artificial diets must be attractive and readily ingested. Diet particles must be of suitable size for ingestion and must elicit a feeding response from the larvae. Diet particles must remain available in the water column
Stability	Artificial diet particles must maintain integrity in aqueous suspension and nutrient leaching must be minimal. Some nutrient leaching may be beneficial in enhancing diet attractability.
Digestibility	Artificial diets must be digestible and their nutrients easily assimilated.
Nutrient composition	Artificial diets must have an appropriate nutritional composition. Material added to the diet as binders or the components of microcapsule walls must have some nutritional value.
Storage	Artificial diets must be suitable for long-term (6—12 months) storage with nutrient composition and particle integrity remaining stable.

Despite considerable progress in recent years, many questions regarding fish larval nutrition remains largely unanswered, and several research avenues remain open. A holistic understanding of the supply line of nutrients is important for developing diets for use in larval culture and for the adaptation of rearing conditions that meet the larval requirements for the optimal presentation of food organisms and/or micro diets.

Indigenous finfish larval feed technology of ICAR-CIBA

Most of the hatchery feeds for finfish and shellfish in Indian market is imported. As the finfish farming is picking up in India, there is an increasing demand for cost effective indigenously developed larval and nursery finfish feeds in India. CIBA beaing a nodal research organisation in the brackishwater sector, is involved in development larval feeds of range of specifications and properties to the different life stages of the finfish larvae for diversified brackishwater aquaculture species of different trophic nature.

Larvi*Plus* :

CIBA is developing series of fish larval feeds as Plus series. These are micro-particulate feeds of different particle sizes (300 to 800 micron) were prepared to contain 55% crude protein and 12% lipid, using premium ingredients enriched with EPA and DHA. The feeds were evaluated in commercial hatcheries, who have signed MoU with CIBA under public private partnership (PPP) mode. CIBA also developed micro bound larval feeds for milkfish and mullet using extrusion cooking. Feeds were formulated to contain 55% crude protein and 15% lipid, using premium ingredients of high digestibility and nutritive value. The larval feeds were tested in in our own research facility and in commercial hatchery facility. The results revealed that the feed have better attractability and palatability for finfish larvae of different stages with respective particle sizes. Feasibility analysis for scaling up of the technology is in progress at CIBA's.

Microbound larval feed

Micrparticulate particulate larval feed