Training Manual on

SEED PRODUCTION AND CULTURE OF BRACKISHWATER CANDIDATE FINFISH SPECIES

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An Overview of induced breeding techniques of Brackishwater finfishes

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Introduction

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

Selection of breeders

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

Sex determination

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

Methods of Breeding

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

Artificial fertilization by striping

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

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

Environmental manipulation

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

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

Induced Spawning

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

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

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

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

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

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

Biology of Milkfish (*Chanos chanos*)

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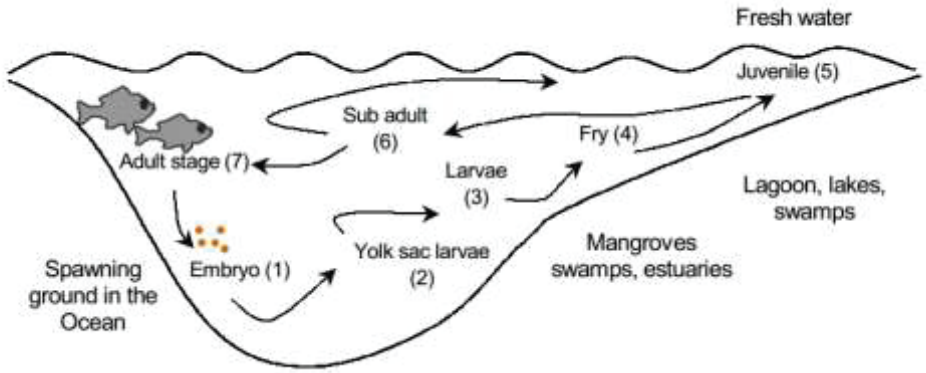
Life history and habitat

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**)**

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.

Seed production of Milkfish (*Chanos chanos*) – Global scenario

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Brackishwater finfish culture depends upon the availability of fry or fingerling for stocking. In past, these seeds were collected from wild sources but with the domestication and fruitful research experience, breeding and seed production was achieved for few finfish species to produce reliable source of seeds. Seed production is an important activity of any hatchery which needs knowledge of fish biology, its spawning behavior, nutritional requirement, heath aspects etc. It also includes activities related to larval biology, nutritional requirement and culture till it attains stock able size.

Culture of Milkfish (*Chanos chanos*) is an age old practice among south East Asian country and in few states of India also. Characteristics like euryhaline nature, rapid growth, herbivores feeding nature are the major reasons which make it an ideal candidate species for culture. Maturation has been reported in varying salinities (0 – 125 ppt). In traditional culture practices, wild caught fry were cultured in different culture systems of fresh water, brackishwater and marine waters. Initial observations on the fry of milkfish in India were made by Panikkar et al. (1952). Adult milkfish from has been reported from CoroMandal Coast, Palk Bay, Gulf of Mannar and Malabar Coasts, Salt pans of Kakinada, Pulicat lake, Vishakhapatnam, Krusadai and Rameswarm Islands, Bakkhali region of lower Sunderban. On the basis of occurrence of adult milkfish, spawning ground has been classified as Madras, Mandapam, Tuticorin, Vizhinjam, Cochin, Calicut zone. Estuaries and mangroves areas were recorded with abundance of fry after spawning season.

Till 1970's source of milkfish seed for nursery rearing and growout culture was wild sources. But Rapid increase in milkfish farms created the need of controlled breeding units for healthy and reliable seed supply. Since that time research was focused in South East Asian countries for breeding in sea cages with the help of hormone manipulation in adult fishes. As the fishes mature in +5 years in case of males and +7 years in case of females, creating a broodbank has been a challenged task. Broodfishes which were captured from sea were maintained in sea cages for spawning.

Adult milkfish are found in open sea are 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, 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 TL 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 for further growth. Milkfish juveniles larger than 20 mm have the characteristic shape and morphology of the adult fish.

Seed production in South East Asia (Philippines & Taiwan)

Milkfish aquaculture in the Philippines has expanded from the traditional culture in brackishwater ponds and freshwater pens to marine cages and pens. 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. Broodstock are kept in deep concrete tank fitted with a recirculating system. An airlift PVC pipe (inset, arrow) fitted with a fine mesh net bag collects eggs naturally spawned by milkfish broodstock. Sex ratio of 2:1 (M : F) is maintained in tanks. Fertilized

eggs are represented by 1.1-1.23 mm oocyte dia. Salmon or Carp pituitary homogenate in combination with HCG, or HCG are used to induce the fishes for spawning. Numbers of hormone injections may be 1-5 (mostly 2) with injection interval of 6 - 24 hour (mostly 8-12 hour). Time to stripping is commonly 6-17 hour (12 hour appears best) in case of oozing female and male. A 5 - 13 kg female can produce 300000 eggs/kg body weights. 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 of milkfish can be done in extensive/intensive systems for 20 – 30 days before initiating nursery rearing. In hatcheries, Indoor larval rearing facilities are utilized for rearing the larvae in green water systems. Rotifer and Artemia nauplii were provided upto 15 – 20 days and weaned to artificial diets later on.

Conclusion

Seed production of Milkfish in hatcheries was achieved in Taiwan which was adopted by Philippines and Thailand for the production of milkfish larvae. In other countries, seed were collected from natural sources. In India, first successful captive breeding was achieved in the year of 2015 which has paved the way for hatchery production in India. Lack of availability of hatchery reared seeds was felt since long back yet the seed production in hatchery has been realized recently.

Broodstock development, Induced breeding and larval rearing of Milkfish (*Chanos chanos*)

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Introduction

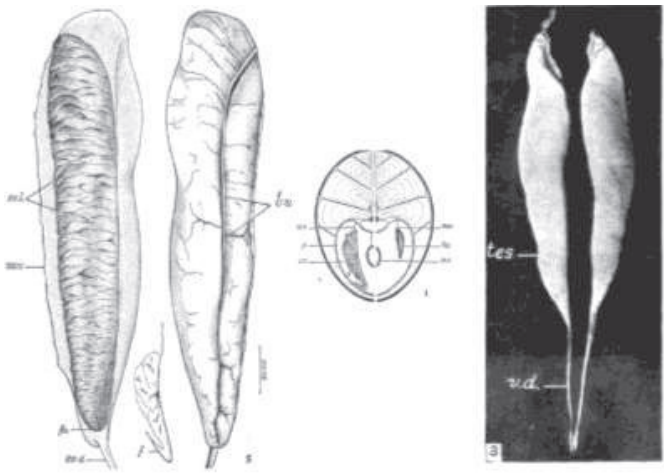
Milkfish (*Chanos chanos*) is traditionally cultured in Philippines. Indonesia also through its small scale hatcheries is a major producer of milk fish seeds. Milkfish culture is also practiced in some Pacific Islands viz. Kiribati, Nauru, Palau and the Cook Islands as well as in countries like Taiwan. 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 4th day after hatching. Critical period is between the 4th and 6th days. *Isochrysis galbana* and *Tetraselmis chuii* in addition to *Chlorella virginica* 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) and first feeding initiated with rotifer *Brachionus plicatilis* from 2 day post hatch (dph). In the LRT, green algae were also added to condition the rearing water. *Artemia* nauplius was introduced along with the rotifer on 14 day post hatch (dph). The milkfish larvae reached to the early fry stage (15mm) on 20 dph.

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 a herbivorous euryhaline species is slowly getting position as an alternative species for shrimp aquaculture in 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 economical 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 in brackishwater aquaculture of India. Milk fish fingerling 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. Wit Milkfish being similar in 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.

Nursery rearing and growout culture of Milkfish (*Chanos chanos*)

Aritra Bera, Babita Mandal, Tanveer Hussain

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.

Grow out farming

1. Pen culture

- ❖ Easily manageable pen can be constructed in shallow areas (5,000– 10,000 m² in size) of estuarine lagoons/ bheries, which are having high natural primary productivity.
- ❖ Water depth in culture areas should not be less than 1 meter. Stocking density of milkfish fingerlings of 40–60 g body weight is 30,000–40,000/ ha which may be stocked once or twice in a year. In pen culture system, milkfish fingerlings feed on naturally available food generally.
- ❖ High stocking density in pen culture can be done using supplemental feed, which can produce 10–20 tonnes/ha of marketable milkfish.

2. Pond culture

A. *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.

3. Modified extensive farming

(Monoculture and polyculture)

- ❖ Existing fish / shrimp farms can be used for modified extensive monoculture farming of milkfish.
- ❖ In non-aerated ponds, based on different water depths in ponds, stocking density and crop yield can be adjusted accordingly:
  - 3 feet water depth : 5,000 fingerling with final harvest of 1 ton/ acre/crop
  - 6 feet water depth : 10,000 fingerling with final harvest of 2 ton/acre/crop
  - 9 feet water depth : 15,000 fingerling with final harvest of 3 ton/acre/crop
- ❖ In this farming system, artificial feed (floating pellets) along with natural feed is provided to gain highest production in short period.
- ❖ Daily feeding should not exceed 25-30 kg of floating pellet/acre in pond having 3 feet of water depth.
- ❖ Marketable milkfish having body weight of 200 - 300 g can be arvested after 3-4 months of culture.
- ❖ Polyculture of milkfish with other brackishwater cultivable species viz. Crab, pearlspot, shrimp and mullets can be done. In Lowsaline ponds, it can be cultured with carps, pangasius, tilapia and freshwater prawn.
- ❖ Carnivorous fishes like seabass, murrels and cobia should be avoided for polyculture with milkfish.
- ❖ In extensive polyculture ponds, stocking density of all the fishes should be according to water depth of culture pond.

4. Intensive farming

- ❖ Intensive farming of milkfish fingerlings in pond having water depth of 0.1–1.5 m lead to more production. 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.

5. Cage culture

- Small milkfish cages can be staked in shallow waters along coastal bays or set-up in deep water with appropriate floats and anchors.
- Fingerlings of 40–60 g body weight are reared in net cages with stocking rates of 5 - 30 fingerling/ m^3.
- Depending on the water quality, milkfish yield in cage culture could be 10-20 kg/cubic meter.

Biology of Grey Mullet (*Mugil cephalus*)

Rekha M.U., Krishna Sukumaran, 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).

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).

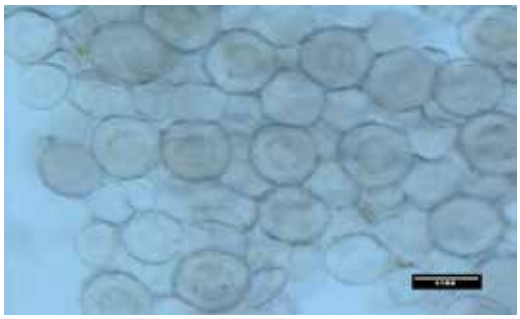
Maturity stages of ovary of *Mugil cephalus*

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

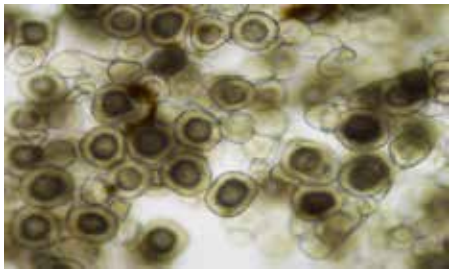
The ovaries appear as reddish, translucent structures united at the posterior end. The entire gonad occupies one fourth of the body cavity. The oocytes correspond to previtellogenic oocytes and are characterized by a small size.

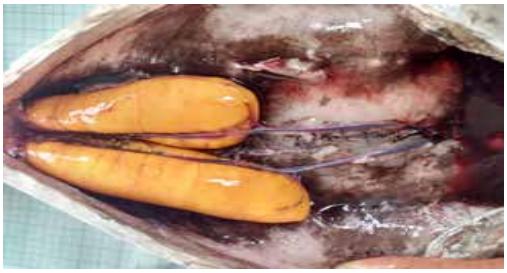


Immature ovary inside the body cavity

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.

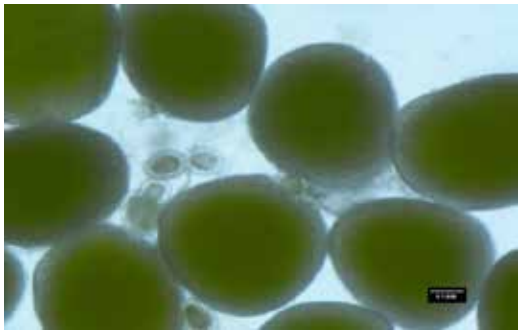




3. Advanced maturation / Mature

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

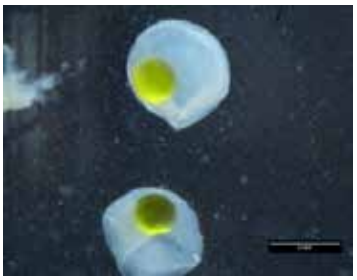


Matured ova are seen under light microscopy. Arrow indicates immature ova

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).

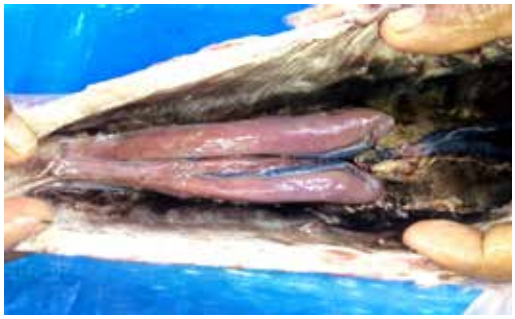
Ripe ovary inside the body cavity



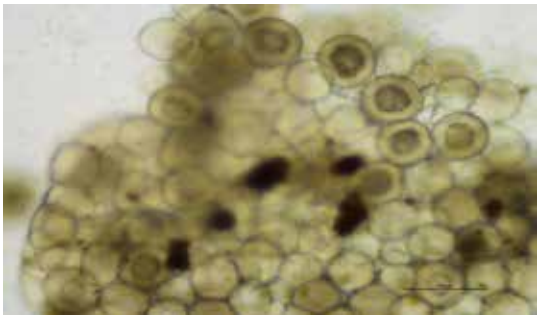
Ripe ova are seen under light microscopy.

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

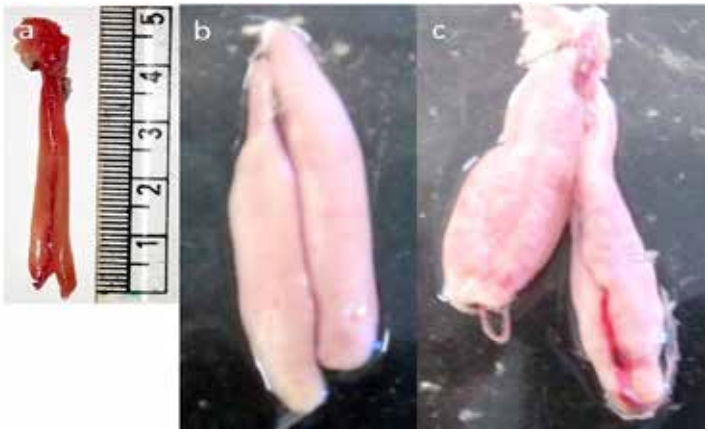
The testis appears as two threads like structures, united at the posterior end. They are semi-transparent and measure about 5 to 7 cm in length. They occupy almost half of the body cavity.

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. The size of the testis is almost similar to that of the previous stage. A 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).

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

Krishna Sukumaran, M. Kailasam, Prem Kumar, Rekha M.U., Dani Thomas, M. Makesh

Introduction

Taxonomy

Class Actinopterygii

Order Mugiliformes

Family Mugilidae

Genus Mugil

Species *Mugil cephalus*

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

Globally in the year 2014, the total production of grey mullets was recorded at 1,51,794 t of which 12,360 t was contributed by aquaculture production. Thus aquaculture made 8.14 % contribution to the total global production of the species, this is significantly low in comparison to the species like Asian seabass where 41.2 % contribution was made by aquaculture towards its total production. The reasons which make a fish an ideal candidate for aquaculture production are related to its seed availability, market value, growth rates, feeding niche and acceptance of artificial feed, adaptability to aquaculture systems, rearing environment and resistance to disease. Given its position in food chain and its good market value, grey mullets have enormous potential for contributing to a sustainable aquaculture model which is the need of the coming decades. It remain to be debated which of the factors whether the seed, feed or the market has to be strengthened to enable the species to realise its true aquaculture potential.

Currently, the largest contributors to the global aquaculture production of grey mullets is Egypt, followed by Republic of Korea, Italy, Taiwan province of China and Israel. Most of the global aquaculture production of grey mullet is reliant on wild seeds. In India, wild seed availability of grey mullets is during the July to August in the west coast around Puduvypu region and around October-November in the east coast of India around Kakinada. Aquaculture of grey mullets in our country is on a limited scale in few pockets. The culture is reliant on wild seed using natural productivity and supplementary feeding

based on agro-bye-products. There is good scope of improving the fish production if consistent hatchery produced source is made available. It is in this context that broodstock development and standardisation of the induced breeding protocols assume significance.

Broodstock Maintenance

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

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

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

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

Environment and water quality

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

Natural feed and formulated feed

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

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

Size at maturity and broodstock selection

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

Reproductive biology of grey mullets

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

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

Hatchery production- Global status

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

Maturity assessment

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

Induction of maturity

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

Induced breeding and larval rearing

On obtaining mature fish with ova diameter exceeding 600 micro-m, a priming dose of 20 mg kg^{-1} of carp pituitary homogenate and a resolving dose of LHRHa, 200 micro-g per kg^{-1} is considered as the best combination for induced breeding via intra-muscular injections. The fish are kept at a ratio of 2-3 males: 1 female for breeding. The fish are reported to spawn 12- 14 h of receiving the resolving dose. The eggs are incubated at around 500 no L^{-1} in aerated tanks. High salinities of above 35 ppt are recommended for incubation of eggs. At 26 ^{0}C an incubation time of 28 h is reported, based on the temperature incubation period of 48- 60 h is also reported. Larvae of grey mullet begin feeding on the third day when they are provided algae and rotifers. Feeding of artemia nauplii is initiated on the seventh day.

Other inducing agents used successfully in different studies

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

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

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

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

Priming dose- 20-70 mg CPE or 10,000 IU HCG and a resolving dose of 200 micro-g LHRHa, ova dia- 570-580 micro-g was reported for successful spawning and fertilisation by Gharabawy and Assem (2006).

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

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Nursery rearing and Grow-out Culture of Grey Mullet (*Mugil cephalus*)

G. Biswas, M. Kailasam, Tanveer Hussain and R. Subburaj and G. Thiagarajan

Introduction

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 fisheries 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.

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

Operational cost (OC)	**Low density FF**		**High density FP**	
Grey mullet fry	15000 @6/-	90000	30000 @6/-	180000
Feed	2000 kg @35/-	70000	-	
Other inputs		30700		55370
Manpower		12500		10000
Sub-total		203200		245370
Interest on OC @ 10% annually				
	For 5 months	8467	For 4 months	8179
Total OC		211667		253549
Return from sale of fingerlings				
Grey mullet fingerlings	12630 nos. @30/-	378900	28290 nos. @20/-	565800
Net return		**167233**		**312251**
Benefit-cost ratio (BCR)		**1.79**		**2.23**

Grow-out culture of grey mullet

Monoculture

Monoculture of grey mullet depends on availability of suitable seeds as stocking materials. Various on-station and out-station trials have proven that monoculture of grey mullet can be an economically viable farming option provided that ponds are stocked with seeds reared initially in nursery. The pond for monoculture is prepared first, following eradication of unwanted organisms and application of manures and fertilizers. Advanced fingerlings of >50 g size are stocked at 10000 nos./ha. Fish are fed with supplementary feed. In an 8-month culture, fish become 500-800 g with total production of 3-4 ton/ ha.

Polyculture

Polyculture is a farming practice where two or more species of fishes are reared together. The concept of polyculture is based on rearing of two or more compatible aquatic species together resulting in higher production compared to monoculture. The underlying goal of polyculture involves increasing productivity by more efficiently utilizing ecological resources within an aquatic environment. Sometimes, one species enhances food availability to other species and thus increases total fish production per unit area. It is commonly believed that polyculture gives higher production than monoculture in extensive and semi-intensive systems and is considered more ecologically sound than monoculture. Before stocking of seeds, pond is prepared well following eradication of pest and predatory fishes, removal of bottom mud and liming, fertilization etc. The ready ponds are stocked with seeds of fish species at 8000-15,000 nos./ ha along with tiger shrimp seeds of 15,000-30,000 nos./ha. The stocking density varies with the quantum of seed availability. Natural pond productivity is maintained by fertilization. In addition, supplementary feed prepared from locally available ingredients can be used at 2-5% body weight daily. This kind of system can yield a total production of 1.5-3.0 ton/ha in 6-10 months. The preferred species among fishes

are: mullets- *M. cephalus*, *Liza tade* (tade grey mullet), *L. parsia* (goldspot mullet), milkfish- *Chanos chanos*, pearlspot- *Etroplus suratensis* and tiger shrimp- *Penaeus monodon*. In an out-station trial, 3 ponds (1 ha each) were stocked with *M. cephalus* (40-50 g size) as the major species at 10000 nos./ha, *L. tade* (10 g size) at 2000 nos./ha, pearlspot (10 g size) at 1000 nos./ha and *P. monodon* at 4500 nos./ ha. Supplementary feeding was provided with floating feed. After 150 days, shrimp with 45 g ABW was harvested and fishes were harvested after 300 days. It resulted in 2.8 ton/ ha production with a net return of Rs. 2.9 lakh/ ha. In another out-station trial, ponds were stocked with *M. cephalus* at 5000 nos./ha, *L. tade* at 5000 nos./ha, *L. parsia* at 10000 nos./ha and *P. monodon* at 40000 nos./ha. After 300 days, total production of 3.6 ton/ ha with a net return of Rs. 5.6 lakh/ ha was achieved.

Conclusion

Major constraint in mullet culture is inadequate availability of seeds. The natural seed availability has become uncertain and sporadic now-a-days. Breeding and seed production of mullets are yet to be achieved in India. Concerted effort on breeding and seed production has been made by ICAR-CIBA since almost two decades and success in breeding will meet up the seed requirement soon.

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Endocrine control of reproduction in brackishwater candidate species

Sherly Tomy, Aritra Bera, Krishna Sukumaran

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.

Organization of hypothalamo-pituitary-gonadal complex in fish

The internal and external cues received by nervous systems are channelled to trigger the endocrine system present in the brain to release hormones to regulate reproduction. The pulsatile secretion of GnRH controls reproductive and sexual function in vertebrates. Many teleosts possess at least two or three GnRH types (GnRH1, GnRH2 and GnRH3). GnRH binds to its cognate receptors located on the pituitary to regulate the synthesis and release of gonadotropins (GTHs): follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In teleost fish, the pituitary is directly innervated by neurosecretory fibres from the brain and releases their neurohormones close to the pituitary cells that synthesize the hormones, thus lacking the hypothalamo-pituitary portal system median eminence observed in mammals. The direct innervation in teleosts may allow a faster and precise control over the secretion of pituitary hormones.

The gonadotropins are transported through blood to the target cells in gonads where it binds with specific receptors in the cell membrane of theca and granulosa cell of ovary or Leydig cell of testis and control gonad development and maturation. They stimulate the production of gonadal steroid hormones including androgens and estrogen which induce development, growth and final maturation of germ cells and their release to the exterior aquatic environment. The differences in steroidogenic potency between teleost FSH and LH are related to the state of gonadal development, whereas FSH and LH have similar steroidogenic potency during early phases of gametogenesis, LH is generally more potent than FSH during late stages of gametogenesis. The effect of GTHs on the release of gonadal steroids will depend on the density of the receptors for FSH and LH in the target cells. The levels of steroidogenic pathway will determine the rate of production of sex steroids that regulate the process of oogenesis and spermatogenesis. Several growth factors such as insulin-like growth factors (IGFs) and activin also mediates sex steroid synthesis. The gonadal steroid hormones also provide positive and negative feedback effects on the HPG reproductive axis. 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.

Recent findings of kisspeptin and gonadotropin-inhibitory hormone (GnIH) added new players in the reproductive system, which stimulate and inhibit mostly GnRH neurons, respectively. In 2000, a hypothalamic neuropeptide inhibitor of gonadotropin secretion termed as gonadotropin-inhibitory hormone (GnIH) was reported from quail which exerts its actions through a putative G-protein coupled receptors (GPR), e.g., GPR147. Besides this action on GnRH neurons, GnIH was shown to cross-talk with the HPG axis at the level of anterior pituitary gonadotropes, which express GnIHR. Moreover, GnIH may also act directly at the gonadal level. GnIHR expression has been reported in the pituitary and the gonads. Kisspeptins, the peptide product of KISSI/Kiss1 gene and its cognate receptor (GPR54) has been proven to play a major role in the regulation of gonadotropic axis, with essential functions in the timing of puberty onset and the control of gonadotropin secretion. The finding of the involvement of KP and its receptor Kiss1r in the initiation and maintenance of reproduction is considered as one of the most important discoveries made in the field of reproductive neuroendocrinology. The presence of two types of kisspeptin encoding genes (kiss1 and kiss2) and two forms of kisspeptin receptor genes (kissr1 and kissr2) has been reported in teleosts compared to mammals. Central as well as peripheral administration of kisspeptin robustly increases systemic levels of the LH and FSH in sexually immature and mature rodents. The stimulatory effects of kisspeptin on gonadotropins are blocked by GnRH antagonists, indicating that the kisspeptin induced stimulation of the HPG axis occurs via the hypothalamic GnRH neuronal network and not directly through Kiss1r in the pituitary. Research data from various vertebrates suggest that kisspeptin is also involved in the mediation of gonadal steroid-negative feedback, estradiol positive feedback during ovulation, as well as seasonal, circadian, metabolic, and stress signals to the reproductive axis. These neurons, containing KP and GnIH, are also expressing receptors of the metabolic hormones. Therefore, this multifaceted neural circuit of hypothalamic neurons can act to integrate alterations in metabolic homeostasis with the neuroendocrine regulation of the HPG axis function.

Reproductive cycle of fish

The fish reproductive cycle is separated in to the growth (gametogenesis) and maturation phase (oocyte maturation and spermiation), both controlled by the reproductive hormones of the brain, pituitary and gonad.

Vitellogenesis and oocyte maturation

The major event during vitellogenesis is the production of the yolk protein precursor (vitellogenin) and its sequestration into the growing oocyte. Final oocyte maturation (FOM) occurs at the completion of vitellogenesis and includes a number of cytological and nuclear changes that prepare the oocyte for ovulation and fertilization. During FOM, the nucleus or germinal vesicle (GV) migrates to the periphery of the oocyte. During or soon after GV migration, coalescence of the lipid droplets and yolk globules occurs, followed by the breakdown of the GV membrane (GVBD) and the re-initiation of meiosis, which was arrested in prophase I during vitellogenesis. The oocyte is ovulated with its chromosomes arrested once again, at metaphase II, and meiosis is reactivated and completed upon fertilization.

Spermatogenesis and spermiation

The gametogenic process in the males is separated into two phases. Spermatogenesis is the first phase and it includes the proliferation of the spermatogonia, the multiplication of the spermatocytes I with multiple mitotic divisions, the production of spermatocytes II with meiotic division and their differentiation to spermatids. The process is completed with the production of flagellated spermatozoa, i.e., spermiogenesis. The spermatozoa are released in the sperm ducts during the second phase of the male reproductive cycle, i.e., spermiation, which occurs during the spawning season. Sperm is ejaculated

spontaneously by the fish and can also be expressed easily from the testes after application of gentle abdominal pressure (i.e., stripping).

Endocrine control of gametogenesis and final maturation

In teleost fish, as in mammals, gametogenesis and final maturation is regulated by the interplay of systemic and intra-gonadal factors and the importance of each type of regulation varies depending on the developmental stage of the gonad. The secretion of the pituitary gonadotropins is regulated by the hypothalamus through multiple hormones, the major one being GnRH. Besides GnRH, other regulatory factors regulating pituitary gonadotropes are kisspeptins and dopamine, hormones such as γ-aminobutyric acid (GABA), norepinephrine, neuropeptide Y (NPY), serotonin, ghrelin, leptin, IGF-I), glutamate etc. Dopamine (DA; in some fishes) and GnIH exerts a negative effect on the functions of GnRH on the pituitary gonadotropes while kisspeptin stimulates gonadotropin secretion. 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.

The pituitary-derived gonadotropins, FSH and LH are primary mediators of gonadal steroidogenesis and gametogenesis. They bind and activate specific receptors (FSH receptor (FSHR) and LH receptor (LHR)), present on the surface of gonadal somatic cells, regulating the expression and activity of key steroidogenic enzymes. FSH is considered to be involved in the initiation and early stages of gametogenesis, such as vitellogenesis and spermatogenesis, to some extent through the synthesis of estradiol-17β (E2) and 11-ketotestosterone (11-KT), respectively. In response to stimulation by E2, the liver produces vitellogenin in females 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, with a transient increase in plasma testosterone (T) during germinal vesicle migration and a dramatic elevation in the plasma levels of maturation inducing steroids (MIS) or maturation inducing hormone (MIH) [17α, 20β-dihydroxy-4-pregnen-3-one (17α,20β-DP) and 17α, 20β, 21-trihydroxy-4-pregnen-3-one] mediated by the enzyme 20β-hydroxysteroid dehydrogenase (20β-HSD)] in several teleost fish which acts at the level of the oocyte to induce FOM (Fig.1). There is a distinct shift in expression of steroidogenic enzyme genes from cytochrome P450 aromatase (P450arom) to 20β-hydroxysteroid dehydrogenase (20β-HSD) in granulosa cells immediately prior to oocyte maturation.

The MIS bind to specific receptors on the oocyte plasma membrane. Recently, a strong candidate for the MIH receptor, membrane progestin receptor (mPR), was identified. In zebrafish, two types of mPRs, mPRα and mPRβ, were identified. 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: a complex of cdc2, the catalytic subunit, and cyclin B, the regulatory subunit) which is responsible for the resumption and completion of FOM and is reflected morphologically by the migration of the germinal vesicle toward the animal pole (GV migration) and GVBD. This is accompanied by the condensation of chromosomes, extrusion of first polar body and meiotic arrest. At this stage the oocytes absorb water and inflate and are released in to the ovarian lumen. Completion

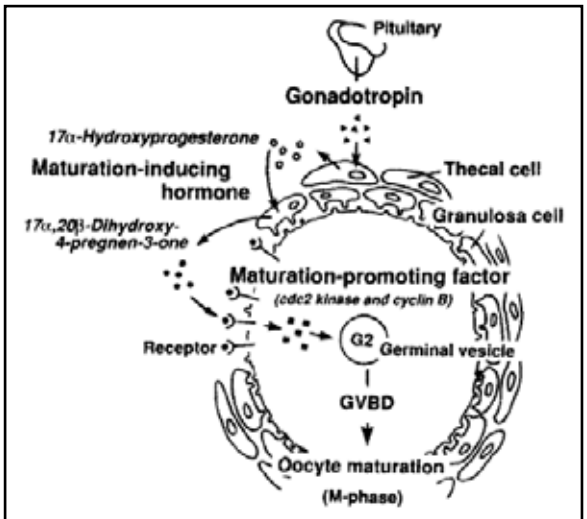

Fig.1. Hormonal Control of Oocyte Maturation and Germinal Vesicle Breakdown (GVBD)

of the meiotic division and extrusion of second polar body is delayed and proceed only if egg is fertilized. In addition to LH, insulin-like growth factor I (IGF-I) has been shown to induce maturational competence and FOM. Depending on the species, the steroidogenic shift described above and the process of FOM may take place over the course of a few hours, a few days, or more than a week.

In males, FSH acts on the Leydig cells to produce 11-ketotestosterone (11KT), a potent androgen in fish. In turn, 11-KT activates the activin B production by Sertoli cells to stimulate premitotic spermatogonia to complete spermatogenesis, mediated also by growth factors line insulin-like growth factor or activing B secreted by the Sertoli cells. Similar to the females, an increase in plasma LH levels at the onset of the spawning season causes a shift in the steroidogenic production of androgen from Leydig cells to the production of maturation inducing steroid (MIS) which regulates sperm capacitation and spermiation (Fig.2). In males, FSH levels are high while LH levels are low during early spermatogenesis. Androgen production (T and 11KT) remains high through the entire spawning period, since spermatogenesis, spermiogenesis and spermiation occur concurrently.

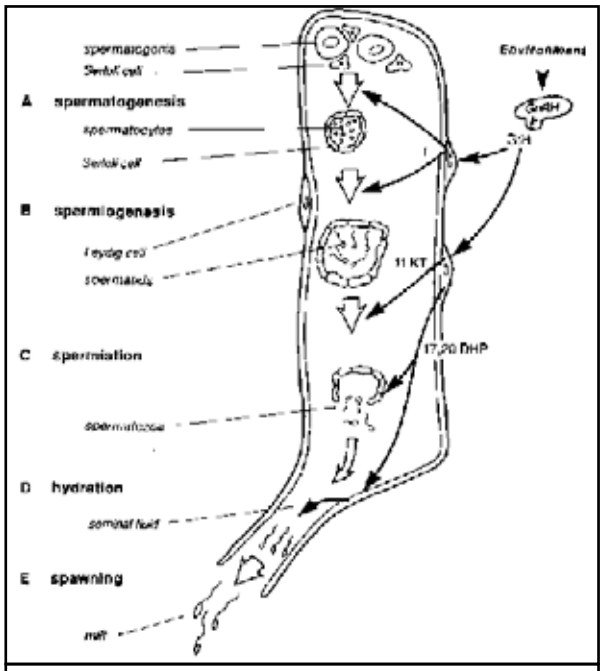

Fig: 2 Testis lobe with progressive stages of maturation showing hormonal control of testis and sperm maturation. Acting through the brain and gonadotropin-releasing hormone (GnRH), environmental cues stimulate the release of gonadotropins (GtH). GtH stimulates Leydig cells to sequentially release the steroid hormones that are responsible for maturation: testosterone (T), 11-keto testosterone (ll-KT), and 17,20-dihydroxy progesterone (17,20 DHP)

Synthesis of steroids involves a complex cascade of oxidative enzymes that convert cholesterol into different functional steroids (Fig.3). The cytochrome P450 11β-hydroxylase, encoded by the CYP11B1 gene is necessary for the final steps of the synthesis of 11-KT whereas cytochrome P450 aromatase (P450arom, encoded by the CYP19A1 gene), catalyses the conversion of testosterone (T) to estradiol (E2). In teleosts, final gamete maturation is initiated by a rapid shift from the synthesis of androgen/ estrogen to the synthesis of MIHs. This steroidogenic shift is typically accompanied by an increase in steroid synthesis. Biosynthesis of steroid hormones has an acute and a chronic hormonal regulation. The circulating E2 also has feedback effects on E2-sensitive cells in the brain and pituitary, and plays a role in regulating the HPG reproductive axis.

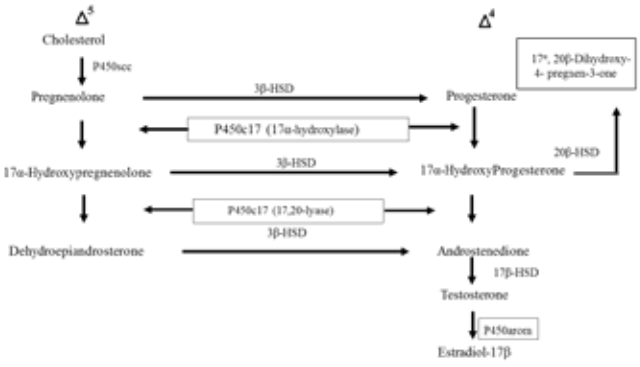

Fig.3: The steroidogenic pathway (Δ4 and Δ5 pathways) involved in the production of 17β-estradiol during oocyte growth (vitellogenesis) and 17α, 20β-DP (oocyte maturation) in the fish ovary

Reproductive dysfunctions -males and females

Most cultured fish 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. Some fish fail completely to undergo vitellogenesis and spermatogenesis when maintained in captivity. These oocytes are too immature to be induced to ovulate using exogenous hormones. The most commonly observed reproductive dysfunction in captive fish, especially marine species, is the unpredictable occurrence or absence of FOM. Studies showed that the failure of fish to undergo FOM in captivity is due to the absence of LH release during the spawning season, even though the hormone accumulates in the pituitary during oogenesis. 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. Spawning does not always result from such hormonal manipulations, in which case the eggs are stripped manually and fertilized artificially. The stimuli for the female to spawn are probably not only of an endocrine nature and may also depend on (a) the existence of the right environment, e.g., water depth, water flow, nest substrate or vegetation, or (b) the presence of the right number of males exhibiting the appropriate spawning behaviour. On the other hand, fish like salmonids undergo vitellogenesis, FOM and ovulation, but fail to spawn their eggs when reared in captivity. Since ovulation is not synchronized among females, the whole broodstock needs to be checked manually for ovulation two or three times a week during the 6-week- long spawning season. Such handling of the fish is laborious and can result in stress, injury, disease and high mortalities.

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.

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

Krishna Sukumaran, Rekha M.U., K.P. Kumaraguru Vasagam

Introduction

Class- Actinopterygii

Order- Perciformes

Family- Cichlidae

Genus- *Etroplus*

Species- *suratensis*

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

Pearlspot exhibits a high degree of parental care and has very low fecundity as compared to other brackishwater fishes. Successful induced breeding of pearlspot has not been reported. These are the main reasons which makes mass scale seed production of the fish challenging. Hence development of technologies which allow seed production at multiple locations in the form of backyard hatcheries or small scale seed production systems is important. However the fish is easier to breed compared to many other brackishwater fish and today different models in a range of systems are available or being tested, so that seed production can be conducted by entrepreneurs, Self- Help Groups or farmers themselves depending on their local resources.

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

i) Seed production of pearlspot in ponds

In pond based experimental trials conducted in CIBA, 50 brooders were stocked in ponds of area, 100 m^2 and depth, 1.2 m after systematic pond preparation (draining, liming, weed fish eradication, manuring for promoting phytoplankton bloom). Additional spawning surfaces as palmyrah leaves tied in bunches to fixed poles, coconut leaf petioles, coconut husks, bricks, pieces of asbestos sheets etc. were provided. Salinity ranged between 15- 30 ppt and transparency was higher than 0.5 m. Brooders

were fed with artificial feeds prepared using groundnut oil cake, rice bran and fish meal fortified with vitamins and minerals @ 2.5%. On observing the presence of hatchings, manuring was done with cow dung @500kg ha^{-1} for enhancing of plankton production. Artificial feed (25-30 g) was also fed once early in the morning. A production of 3500 fry was observed in a year from 5 sets of breeding (Abraham and Sultana, 1995)

ii) Breeding of pearlspot in RCC tanks

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

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

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

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

vi) Seed production of pearlspot in tank based recirculation system

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

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

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

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

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

Conclusion

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

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Induced breeding, larval rearing and seed production of Asian seabass (*Lates calcarifer*)

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Introduction

Successful aquaculture largely depends on the availability of sufficient quality seed at the required time. Availability of quality seed from natural sources is always erratic and undependable. Moreover collection of wild seed will deplete the natural fishery. Almost all of the cultivable brackishwater finfishes do not breed in captivity even though they attain gonadal maturity. Hence it has become necessary to go for induced breeding either by reproductive hormonal or environmental manipulation.

Asian Sea bass (Lates Calcarifer) known as Bhetki is one of the commercially important finfish species caught from inshore areas, estuaries, backwaters, lagoons and fresh water ponds. Sea bass is a fast growing species with ability to tolerate wide fluctuations in environmental conditions. It fetches fairly good price in the domestic market and there is a scope for export to Middle East and some of the European countries. It is considered as a potential candidate species for farming in saline or freshwater environments in ponds and cages. It is extensively cultured in South East Asian Countries like Thailand, Malaysia, Singapore and Indonesia and in Australia. However in India sea bass farming is very much limited. It is done traditionally in the coastal ponds, this method of farming is neither advisable nor dependable and the availability of quality seed in adequate quantity is one of the major reasons hampering seabass farming in India in a large scale.

Seed being one of the major inputs for a sustainable farming, development of techno economically, viable indigenous technology for large-scale seed production of sea bass has been taken up on priority at the Central Institute of Brackishwater Aquaculture, Chennai and a technology package has been developed.

Biology of Sea bass

Sea bass is an euryhaline species capable of withstanding wide salinity fluctuations. It is a catadromous fish migrating towards sea for breeding. It is a fast growing fish and suitable for culture in marine, brackish and fresh water bodies.

Seabass grows in salt water and fresh water, it migrates to sea in the reproductive phase. Seabass spawns in the sea not far away from the shores but of about 20-30 kms. The newly hatched larvae are drifted into coastal waters and even enter fresh water areas through water currents

In sea bass, sexes are separate; however, they are protandrous hermaphrodite. Most of the fishes are males when they are small less than 3 kg and change to females when they grow more than 4.0 kg. Seabass is a protracted spawner (i.e an individual fish can spawn two or three times in a season).

SEED PRODUCTION TECHNOLOGY

For sustainable captive seed production the following are important requirements:

- Broodstock fishes
- Broodstock holding facilities
- Facilities for pumping quality seawater
- Maturation and spawning facility
- Egg Incubation
- Live feed culture system-Stock culture and mass rearing facilities
- Larval rearing facilities and
- Nursery rearing

Broodstock Development

The success of seed production depends upon the development of viable captive Broodstock and for a sustainable hatchery operation a viable land based brood stock has to be developed.

Fish Collection and transportation

Fishes in the size range 1.5 kg to 3.0 kgs for males stock and 4 to 10 kg for female stock can be collected either from wild sources or from the culture ponds. The fish should be healthy without external or internal injuries and free from pathogens. If the sources of collection is very near this hardy fish can be transported safely using open containers and from far away places, water tanker with oxygen bubbling is advisable with biomass @ 3-4 kg/m^3 for a transportation duration of 8-9 hrs under water temperature of 27-28^oC.

Acclimatization

Seabass though in general a sturdy fish capable of withstanding environmental variations, the broodstock fish should be kept captive condition with minimal stress to the extent possible. As they are predaceous preferring to feed on live fishes and crustaceans should be acclimatized to feed on inert diet. Fishes either procured and transported have to be 'conditioned' before they are transferred to holding tanks/cages. Prophylactic treatments with fungicides, bactericides are done to avoid manifestation of diseases. Fishes should be screened for pathogens and quarantined for a period 7-10 days before they are transferred to brood stock holding facilities.

Broodstock Holding Facilities

Broodstock can be maintained in larger tanks or in cages or earthen ponds where good quality seawater can be supplied. These facilities should have adequate water exchange system and aeration. RCC circular or rectangular holding tanks of 12 x 6 x 2 m are desirable.

Broodstock Holding Tanks

Broodstock Maintenance

Under captive conditions Broodstock fishes can be maintained at a density of 1 kg/m^3. Fishes in the size group of 1.5 to 3.0 kg to cater the need of males and 4 to 10 kgs for females are maintained.

Water Quality Management

In the Broodstock holding tanks, adequate water exchange to an extent of 70-80% daily is desirable. Since, Broodstock fishes are maintained specifically for maturation and spawning required parameters stimulating the conditions prevailing in the sea would be very much helpful to obtain better response. The following water quality parameters are desirable for maturation and spawning.

TABLE: 1 WATER QUALITY PARAMETERS REQUIRED FOR MATURATION

1.	Water Temperature	28 – 30°C
2.	Salinity	28-32 ppt
3.	PH	7.8 – 8.2
4.	Dissolved Oxygen	< 5 ppm
5.	Ammonia	> 1 ppm
6.	Phosphate	> 60mg/l
7.	Alkalinity	>100 ppm

Management of Feeding For Broodstock

Nutritionally balanced diet is an essential component in broodstock development since the health of brood stock will only determine the health of their offsprings. Seabass is being a voracious carnivorous fish in feeding namely in live fishes/shrimps seabass it may be reluctant to feed on inert feed. However, they can be weaned to feed an frozen fish. To start with fishes are fed with live and frozen fishes and slowly live fish is replaced completely with frozen feed. Frozen fishes like oil sardines and *Tilapia* are fed @ 5% of the body weight of the biomass daily.

Health Management of Broodstock

The most common problem that would be associated will be the 'pathogens'. Common pathogens like *caligus sp, Lernonthropsis sp* and the monogenic trematode D*iplectnum latesi* are encountered in fishes during months of October to February.Broodstock fishes maintained in captivity will be

susceptible for stress. Pathogens inside the holding tanks will infect leading to mortality. Treatment with 1 ppm organo-phosphorus Dichlorvos for one hour if the parasitic infestation is high or 100 ppm formalin in the case of lesser infestation is found to be effective in controlling the parasite infestation.

Maturation and Spawning

Fishes maintained with proper water quality management, feeding and health management attains maturity under captive conditions normally during breeding season. For eg. at Chennai fishes attain full maturation during the months of May to November provide the salinity regime is more than 28 ppt. If the salinity reduces resorbtion of gonad is observed under captive conditions indicating salinity is the major factor influencing the maturation. To evaluate the maturity stages ovarian biopsy using cannula is done. The polythene cannula of 1.2 mm dia is inserted into the oviduct and gently aspirated. The ova is then observed under microscope.

Seabass is a protracted spawner. An individual fish will have in the ovary eggs in different diameter. This facilitates the fish to spawn more than once in the spawning season. Females with egg size more than 0.450 mm is selected for induction of spawning. In the case of males, if thick white viscous milt is seen by gently pressing abdomen such fishes are selected.

Induced Spawning

Seabass fish spawns spontaneously if the conditions could be stimulated as prevailing in the sea. But it may not be that simple. In such cases, the hormone responsible for ovulation in administered extraneously. For Seabass breeding the hormone LHRH-a, a product of SIGMA is highly effective. Female fishes with ova size more than 0.450 mm and oozing males are selected and a single dose of LHRH-a hormone is administered intramuscularly @ 60-70µg/kg body weight for females and 30 -35µg/kg body weight for males preferably in the early hours of the day.

For Example

If the 1mg vial is dissolved in 5ml; Each ml will have hormone concentration of 200 µg. If the selected female fish weighs 6 kgs, at the dosage level of @ 70 ug/kg body weight, the hormone requirement will be

$$70 \times 6 = 420 \text{ µg for female fish}$$

go from the vial $\frac{1}{200} \times 420 = 2.10$ ml has to be drawn for giving injection

For the males – if the weight of male is 3 kg each the requirement at a dose rate of 35 µg/kg body weight will be $35 \times 3 = 105$ µg.

Then $\frac{1}{200} \times 105 = 0.525$ ml has to be injected to each male.

Hormone is taken in separate syringes for injection to female and male fishes

The sex ratio is maintained Female : Male @ 1:2. spawning takes place simultaneously sly after 30-36 hrs after the injection. Spawning takes place normally in the evening hours. For better spawning and fertilization full moon or new moon days are preferable. Being a batch spawner seabass spawns during subsequent days also. The fecundity normally ranges from 0.7 to 3.0 million under captive conditions in a single spawning. The fertilized eggs will measure about 0.78 to 2.0 mm in size and float

on the surface and the unfertilized opaque eggs will sink at the bottom. The fertilization rate depends upon the conditions of the spawner, which is upto 90% under captive condition.

Egg Collection and Incubation

The floating fertilized eggs can be collected by air drifting method or by scooping method or by over flowing method using suitable mesh size cloth. The fertilized eggs are transferred to the incubation tanks for further development and hatching and lesser at density of 70-100 nos eggs/l in the incubation tanks. The eggs will hatch out in 17 – 18 hours after fertilization undergoing developmental stages as follows

Embryonic development Stages	Duration
One Cell stage	30 minutes
Two Cell stage	40 minutes
Four Cell stage	45 minutes
Eight Cell stage	60 minutes
Thirty two Cell stage	2 hrs
Sixty four Cell stage	2 hrs 30 minutes
128 Cell stage	3 hrs
Blastula stage	5 hrs 30 minutes
Gastrula stage	6 hrs 30 minutes
Neurula stage	8 hrs
Early embryo	11 hrs
Heart functional and tail movement	15 hrs
Hatching	17 – 18 hrs

Undergoing different stages of development, the fertilized eggs hatch out after 15-17 hrs. The hatched out larva of seabass measures about 1.2 to 1.4 mm in size with comparatively small yolk sac. The hatching rate depends upon many factors like health of the spawners, fertilization rate, water quality etc. Normally it is observed to be up to 85% under captive condition.

Larval Rearing

Healthy larvae are transferred to rearing tanks and the density is maintained around 20-30 nos/l. The larvae subsist on the yolk for 3 days. From day 4 larvae have to be fed with quality feed. The most preferred feed for early larval stages, is the rotifer (*Brachionus plicatlis* or *Brachionous rodentiformis*), the details of live feed to be given for seabass larvae is given below:

Algae	Green unicellular algae like *Chlorella* sp *Tetraselmis* sp *Nannochlorpsis* or *Isochrysis* sp are needed for feeding the live feed (zooplankton), Rotifer and for adding to seabass larval rearing tanks for water quality maintenance.
Rotifer	Rotifer (*Brachionus plicatilis*) or B.rotundiformis is the most prefereed diet for the fish larvae in their early stages. The size of the Rotifers vary from 50 – 250 µm. The early stage larvae (upto 7 days) are fed with small sized rotifer i.e. less than 120µm and later assorted size rotifer can be fed.
Artemia	Brine shrimp, *Artemia* in nauplii stage are required for feeding the larvae from 9th day. Artemia with its natural nutrient profile required for larval development of fish is used in all the hatcheries. *Artemia* cyst are kept for hatching and the freshly hatched nauplii are given as feed for fish larvae upto 21 days and afterwards *Artemia* biomass can be given.

During the process of rearing the most important aspect to be looked in to the grading seabass by nature is showing differential growth and hierarchy is maintained. The larger ones (shooters) always choose the small and pray upon because of the cannibalistic behaviour. Few large ones will finish of many smaller ones. Hence from day 15 th onwards as frequent as possible 'grading' should be done so that uniform size fry are reared together. Water exchange is to be done to the extent of 30-40%, the bottom debris should be removed periodically, appropriate water quality should be maintained in the larval rearing tank, with technology available with CIBA a survival rate up to 47% could be achieved with average survival rate around 10% during larval rearing phase. A 25 days old fry will be around 1.2 cm in size.

Nursery Rearing

Nursery rearing is the intermediate phase between hatchery and grow out system. Sea bass (*Lates Calcarifer*) can be cultured in ponds or net cages. Before stocking in growout culture system seabass larvae reared in the hatcheries have to be further reared for a period of 30 – 45 days till they attain a size that can withstand changes in the culture systems. In the nurseries, the fry can be stocked in higher densities and reared. This would save space and time in growout phase. The stockable size of the seed desirable is 5 – 10 gm. Nursery can be done in hatchery using FRP or cement tanks, fixing hapas in ponds and stocking in small nursery ponds. The nursery rearing phase will be 30-45 days, during the phase of nursery rearing in tanks and hapas grading of larvae should be done to avoid cannibalism and better survival rate.

Brackishwater Ornamental Fish Breeding Techniques

S.N.Sethi, Babita Mandal, Aritra Bera and G.Biswas

Aquatic ornamental industry is today 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 bulk of the ornamental fish traded constitute of the freshwater fish (almost 90%), however in terms of value their marine counterparts contribute significantly higher. A notable difference is that the freshwater species are mostly captive bred (approximately 90%) and that the marine species are collected from wild, 90- 95% (Oliver, 2001). USA, Europe and Japan are the largest international markets for ornamental fish; 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. India can categorise its ornamental fish species being traded into two categories; the exotic ornamental and native fish of India. The exotic with almost 288 varieties dominates the domestic market. One hundred and eighty seven indigenous species are traded from India. The wild catches form the bulk of the exports (85%) as compared to the cultured ones (Rani et al., 2013). The ornamental fish market has been showing steady improvement in India with the export values touching USD 3.8 million, a growth rate of 14.4 % has been recorded in the ornamental fish export. India's favourite export destination is Singapore (42.85%), followed by Japan (13.88%) and Malaysia (9.97%). Freshwater fish dominate the scenario of cultured ornamental fish species; Molly, Guppy, Platy, Swordtail, Barbs, Cichlids, Angels, Siamese fighter, Tetras, Gold fish, Manila carps, and Sharks (Ghosh et al., 2000). 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 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 Brood/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.

I) Spotted Scat, *Scatophagus argus* L. 1766

Taxonomy Position

Class- Actinopterygii

Order- Perciformes

Family- Scatophagidae

Genus- *Scatophagus*

Species- *argus*

The species is distributed in the Indo-Pacific, Southern India, Sri Lanka, Southern Japan and Tahiti. It inhabits coastal muddy areas, lower courses of rivers and mangrove areas. The fish is an omnivore feeding on detritus, filamentous algae, phytoplankton, macrophytes and zooplankton. It attains a maximum total length of 380 mm. Females of the species are reported to mature at 7-8 months at 150 g size while males mature at relatively smaller size (Barry & Fast, 1992). Care has to be taken during handling of the species due to its venomous spines on the dorsal, anal and pelvic fins which can cause pain for long hours.

CIBA has successfully developed protocols for captive maturation and induced breeding of spotted scat (Kailasam and Arasu, 2011). Brood fish (up to 250- 300 g) size were developed in ponds and tanks by providing optimal environmental conditions and feed for accelerating maturation. Successful spawning of the captive broodstock was achieved by hormonal manipulations. A female fish weighing 200 g with ova diameter of 426 µ was selected and administered with Human Chorionic Gonadotropin (HCG) hormone as a prime dose, followed by Luteinizing Hormone and Releasing Hormone (LHRHa), as a resolving dose. Male fishes were also administered with the same hormones. Forty-eight hours after the treatment, fishes responded and ovulation was observed. Ovulated eggs and milt were stripped from fishes and fertilization was facilitated externally. Larvae hatched out after 19 hours of fertilization, and average size of the larva was 1.62 mm. Larvae were fed with rotifers from day 3, up to day 10, and afterwards with brine shrimp, Artemia nauplii up to day 25, till they reached 7-9 mm size. Fry were weaned to formulated feed and reared further. The hatchery produced juveniles were supplied to entrepreneurs for further propagation. The fish fetches a domestic market price of Rs 30-40 per unit (2 inch size).

II) Silver Moony fish, *Monodactylus argenteus*

Taxonomy Position

Class- Actinopterygii

Order- Perciformes

Family- Monodactlylidae

Genus- *Monodactylus*

Species- *argenteus*

The species is distributed in the Indo-Pacific region. It inhabits bays, mangrove estuaries, tidal creeks. The fish feeds on plankton and detritus. It attains a maximum total length of 270 mm. Males and female fish, TL 50-55 g size were observed to be in mature stage (Prem et al., 2014). At CIBA broodstock of the fish is being maintained in ponds with commercial feeds and captive maturation has been attained in the fish also. Further trails are in progress for captive breeding of the species (CIBA ANNUAL REPORT, 2013-14). Silver Moony fish fetches over Rs 100 per unit in the domestic market.

III) Green Chromide/Pearl Spots, *Etroplus suratensis*

Taxonomy Position

Class- Actinopterygii

Order- Perciformes

Family- Cichlidae

Genus- *Etroplus*

Species- *suratensis*

The species is naturally distributed in the southern India and Sri Lanka. It inhabits freshwater and estuarine water bodies. The fish is an omnivore feeding on detritus, aquatic macrophytes and filamentous algae. It attains a maximum total length of 400 mm. Length at first maturity has been reported as 195 mm in males and 200 mm in females (Bindu et al., 2014). Fecundity of pearlspot varies from 500 to 7550 (Vijayaraghavan et al., 1981; Bindu, 2006). CIBA has developed seed production of pearlspot different systems; ponds, tanks, cages.

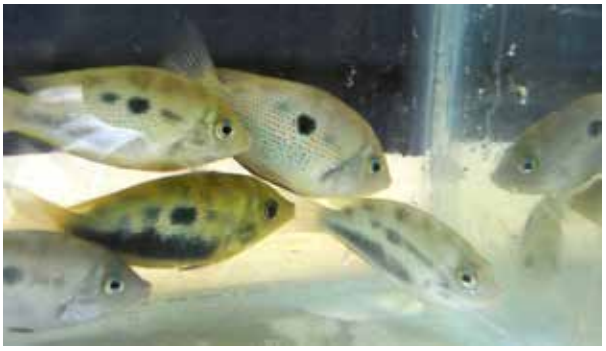
Pond based system: In ponds, area, 100 m2 and depth, 1.2 m, salinity, 15- 30 ppt, 50 brooders was stocked after systematic pond preparation. Additional spawning surfaces were introduced in the pond for egg attachment. Feeding was done using formulated feeds. On observing the presence of hatchlings, manuring was done with cow dung @500kg ha-1 for enhancing of plankton production, artificial feed (25-30 g) was also provided. A production of 3500 fry was observed in a year from 5 sets of breeding (Abraham and Sultana, 1995).

RCC tank system (20 t): In RCC tank, continuous water flow through was provided. Half of the tank bottom is provided with a soil base for egg attachment earthen tiles and hide outs were provided. The tanks are stocked using mature pearlspot brooders at a stocking density of 20 fish per tank at a male female ratio of 2:3. Pair formation and breeding occurs naturally. Fry were collected at regular monthly intervals by lowering the water level. A production of 1200-3500 seed per batch was obtained regularly (CIBA Annual Report, 2010-11).

RAS based tank system (1 t): Breeding trials of pearlspot breeding were conducted in one ton rectangular plastic tanks provided with a continuous water flow using a biofilter facility. Each tank was provided with a small plastic tub filled with clay soil to facilitate breeding. The breeding tank was stocked with 3-4 mature brooders. After breeding the eggs were attached to the sides of the plastic container and the brood fish were observed to take turns in defending the eggs. Larvae were separated and reared using alternate live feeds, Rotifer, *Brachionus plicatilis*, *Artemia* nauplii and by co-feeding with commercial larval diets. One of the most promising results obtained was the production of 8000 larvae by a single pair stocked in six breeding at an average breeding interval of 17.6 ±1.12 days and an average larval number per spawning of 1333.3±143.0.

Seed production of pearlspot in hapas: Seed production of pearlspot were conducted in hapas set in ponds having gentle water flow, salinity- 25-30 ppt. Brooders were maintained in small cages on commercial fish feed. Hapas (1x0.75x1 m) were fixed by casurina poles and clay soil in small plastic tubs were suspended at 0.5 m depth to facilitate nest building. Just above the soil surface 1-2 ceramic tiles are suspended to facilitate egg attachment. Each cage was stocked with 3-4 brooders. Efforts at pair formation are usually observed a few hours after release of fish in the cages. In the initial trials hatchlings were collected from pits in the cage and subsequent larval rearing was practiced, following this method a seed production of 1000-1500 seed per hapa could be observed. However, it is not always possible to observe larvae due to turbidity in the pond. In majority of seed production trials, seed were reared within hapa with parental care. A production between 200-300 numbers of seed (avg. TL, 28.11±1.49 mm; Average Weight; 0.66±0.04 g) was observed per cage within 2- 2.5 months.

IV) 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.

Attached Eggs and two days old larvae of Orange Chromide, *E. maculatus*

Two months old larvae of Orange Chromide, *E. maculatus*

V) 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: 25-26^{0}C; 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).

1-Month Old Fries of *T.jarbua*

2-Month Old Fries of *T.jarbua*

3-Month Old Fingerlings of *T. jarbua*

VI) Banded Chromide , *Etroplus canarensis*

Taxonomy Position

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. This is an endangered species under IUCN lists.

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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Culture of live food organisms - Micro-algae, Rotifer and Artemia nauplii

Babita Mandal, K.P. Sandeep, Rekha M. U., R. Subburaj, K. Karaian

Newly hatched finfish larvae are inefficient to catch and chase their food due to underdeveloped vision and other sensory senses. Larvae hatch out with yolk reserve which lasts for 3 - 4 days, after which exogenous feeding should start. After mouth opening, larvae can start feeding but feed particle size should be smaller than mouth gape. Hitherto, naturally available live feed are nutritionally found best with smallest size to suit the feeding requirement of larvae. Adequate quantity of these live feed should be present during larval rearing phase for the successful larval rearing. Hence, pure culture of these naturally available live feed are cultivated in mass to cater the requirement with adequate nutrition intact.

Pure culture of unicellular microalgae viz. *Chlorella sp., Nannochloropsis sp., Isochrysis sp.* and *Tetraselmis sp*. are maintained in laboratory conditions to avoid contamination which further used for culturing rotifers and copepod. Artemia nauplii are hatched out from cyst. Live feed culture should be started with adequate facility prior larval production to avoid any disruption in supply for larval rearing.

1. Culture of Microalgae

Mass-cultured microalgae are the primary food source for larval and juvenile bivalves, and for the larvae of many crustacean and fish species in mariculture. They also are the primary diet of zooplankton reared as food for late-larvae and juveniles of some crustacean and fish species. Microalgae varied in their proportions of protein (6.6-52%), carbohydrate (5.5-23%) and lipid (7-23%). All species had similar amino acid composition, and were rich in the essential amino acids.

a. Algal culture techniques

Algae 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 available for indoor and outdoor cultivation. (Guillard's F/2 medium, Walne medium etc). There are five different stages in the algal growth.

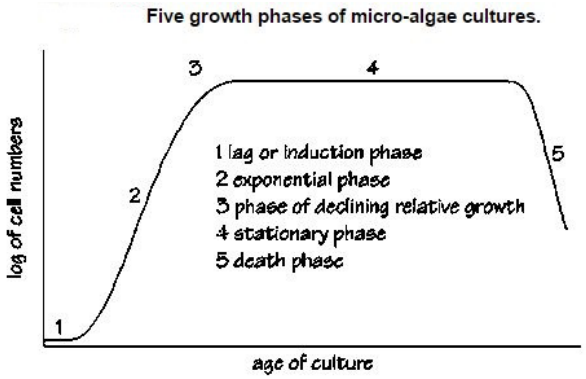

Fig: 1. Different phases in the microalgal life cycle

Indoor culture allows control over illumination, temperature, nutrient level, contamination with predators and competing algae, whereas outdoor algal systems make it very 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. The latter makes it impractical for commercial operations. These are the three basic types of phytoplankton culture which will be described in the following sections.

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.

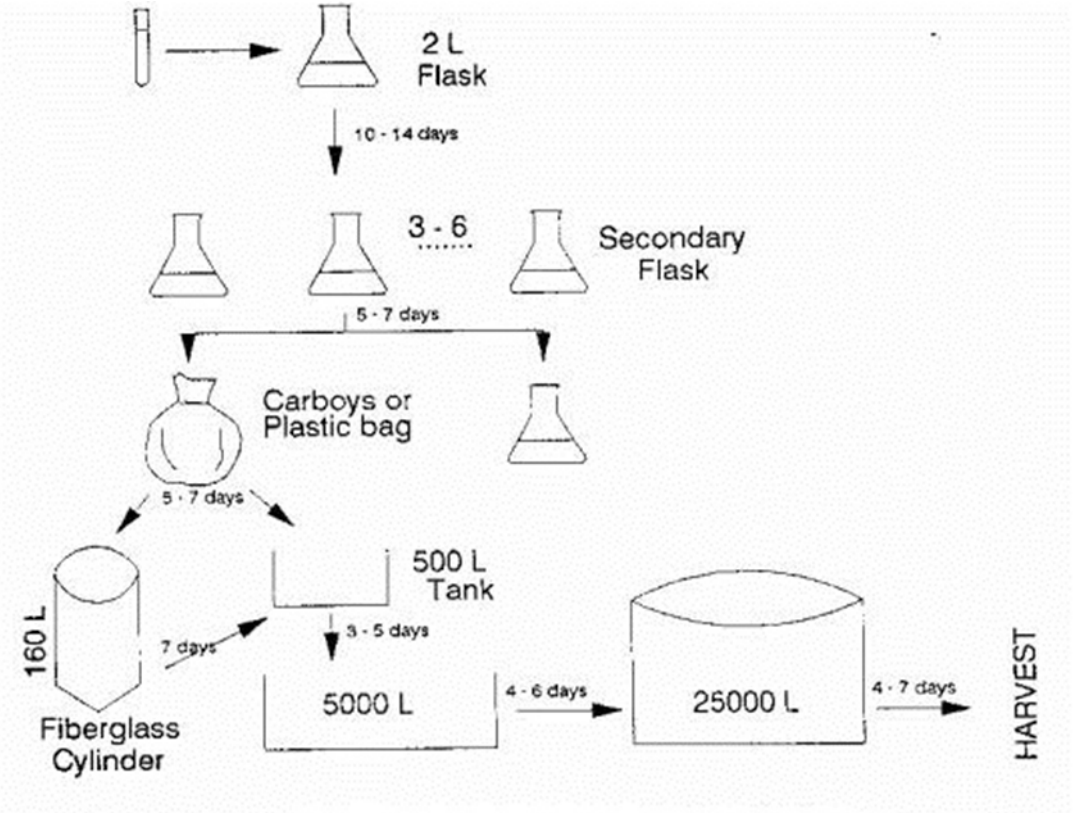

Fig: 2. Diagrammatic representation of batch culture of microalgae

(*Source: FAO fisheries technical paper: 361*)

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.

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

b. 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.

c. Commonly used media for indoor microalgae culture

f/2 Medium		
Stocks		**Per Litre**
1.	$NaNO_3$	75 g
2.	$NaH_2PO_4.2H_2O$	5.65 g
3.	Trace elements (chelated) Na_2 EDTA $FeCl_3.6H_2O$ $CuSO_4.6H_2O$ $ZnSO_4.7H_2O$ $CoCl_2.6H_2O$ $MnCl_2.4H_2O$ $Na_2MoO_4.2H_2O$	 4.16 g 3.15 g 0.01 g 0.022 g 0.01 g 0.18 g 0.006 g
4.	Vitamin Mix Cyanocobalamin (Vit B_{12}) Thiamine HCl (Vit B_1) Biotin	 0.0005 g 0.1 g 0.05

Walne's Medium		
Stocks		
Trace metal solution		**Per 100 ml**
1.	$ZnCl_2$	2.1 g
2.	$CoCl_2.6H_2O$	2.0 g
3.	$(NH_4)6Mo_7O_{24}.4H_2O$	0.9 g
4.	$CuSO_4.5H_2O$	2.0 g
	Nutrient Solution	**Per litre**
5.	$FeCl_3.6H_2O$	1.3 g
6.	$MnCl_2.4H_2O$	0.36 g

7.	H_3BO_3	33.6 g
8.	EDTA	45.0 g
9	$NaH_2PO_4.2H_2O$	20.0 g
10.	$NaNO_3$	100 g
	Vitamin Solution	**Per 100 ml**
11.	Vitamin B_{12}	10.0 mg
12.	Vitamin B_1	10.0 mg
13.	Vitamin H	200.0 µg
	Working medium	
	Nutrient solution	1.0 ml
	Trace metal solution	0.5 ml
	Vitamin solution	0.1 ml

2. Culture of Rotifers

Rotifers are also known as wheel animalculae 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* etc. by movements of corona. In commercial finfish hatcheries they are cultured on mass scale due to their small and preferred feeding size (100 – 280 µ) by larvae. 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.

a. 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

In marine or brackishwater finfish hatcheries *Brachionus plicatilis, Brachionus rotundiformis* are the species which are cultured widely.

b. Specific requirements to start the mass propogation:

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

c. 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 upto 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.

d. Mass culture of rotifer

Clean the rotifer culture tans, add *Nannochlropsis* culture at a density of 20 X 10^6 cells/ml. Inoculate the tank with pure culture of rotifer to achieve an initial density of 10 individual/ml. Allow 7 – 8 days for rotifers density to increase. Harvest and concentrate using a 48 µ mesh plankton net. After each harvest, thoroughly clean tanks with fresh water. Culture of live feed should be scheduled to ensure daily harvest for uninterrupted production. Rotifers can produce asexually, because of the short life span and better nutritive value. This can be achieved by regulating feed, water, temperature, salinity and aeration during the culture process.

e. Rotifer enrichment

The nutritional value 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. Rotifers submerged in these algae (approximately 5×10^6 algae/ml) are incorporating the essential fatty acids in a few hours and come to equilibrium with a DHA/EPA level above 2 for rotifers submerged in Isochrysis and below 0.5 for Tetraselmis. However, the culture of microalgae as a sole diet for rotifer feeding is costly due to the labour intensive character of microalgae production. Most of the time the rotifers are boosted in oil emulsions and fed to the predators which are kept in "green water". This "green water", consisting of 10^6 algal cells/ml (Tetra-selmis, Nannochloropsis, or Isochrysis) is applied to maintain an appropriate HUFA (but also other components) content in the live prey before they are eventually ingested by the predator.

3. Culture of Artemia nauplii

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 marine as well as freshwater 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.

a. Production of artemia nauplii: Environmental parameters

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

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

b. Decapsulation of cyst

The hard shell and chorion should be removed to achieve the hatching of nauplii. Cyst 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 will become orange in color, aeration should be stopped. Cysts are washed with Sodium thiosulphate solution for less than a minute to remove any residual chlorine. Cyst are rinsed with water and kept for incubation.

c. Hatching and collection

A transparent or translucent cylindroconical tank with a valve at bottom is used for hatching purpose. Strong aeration is supplied through open aeration line down to the tip of the conical part of the tank. Filtered natural seawater is used for hatching. Minimum illumination of 2000 lux at the water surface is provided during incubation for a period of 20 – 24 hour. After complete hatching, aeration is stopped. All other light sources are turned off and a light is provided near bottom outlet for nauplii collection. After 10 – 15 minutes, nauplii can be collected with the help of fine mesh net.

Conclusion

For producing healthy seeds, availability of suitable live feeds during rearing of different larval stages is suggested for profitable aqua farming. Apart from long known live feeds, another live feeds can be tried first on experimental basis for finfish larval survival. This may help to reduce cost of production as well as labour intensive process.

Health management in Brackishwater finfish hatchery

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Health management in hatcheries is essential for the successful operation of the hatchery. 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 and low survivability. Prevention is considered as the first line of defence against 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 is 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 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 animals 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. 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 hatcheries.

Bacterial diseases

Aeromonad septicemia

Aeromonad septicaemias are caused by *Aeromonas hydrophila* and to a 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 health 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. 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 of seabass causing up to 100%

mortality. 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.

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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Development of formulated feed for cultivable brackishwater finfishes

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Introduction

Aquaculture farming has shown phenomenal growth in the last decade in India producing protein rich health food and earning valuable foreign exchange. Feed is a major input in fish farming. The development of nutritionally balanced feed involves understanding the dietary requirements of candidate species, selection of feed ingredients, formulation of feeds and appropriate processing technology for producing water stable pellet feeds. Depending upon the type of farming, a wide range of feeds is used for feeding stocked shrimp and fish. While no feed is used in traditional farming systems, supplementary and balanced feeds are used in extensive and semiintensive aquaculture.

All animals including fish requires 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.

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

Protein

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

Protein is required in the diet to provide indispensable amino acids and nitrogen for synthesis of non-indispensable amino acids. A deficiency of indispensable amino acid creates poor utilization of dietary protein and hence growth retardation, poor live weight gain and feed efficiency. In severe cases, deficiency reduces the ability to resist diseases and lowers the effectiveness of the immune response mechanism. Experiments have shown that tryptophan deficient fish become scoliotic, showing curvature of the spine, and methionine deficiency produces lens cataracts.

Protein requirement vary with the age of the fish. Younger animal generally require higher levels of protein (5-10% more protein) than older animals. Carnivores require high dietary protein (40-50%) than omnivores (25-35%). Among the brackishwater finfishes, requirement of protein for Asian seabass

(*Lates calcarifer*), milkfish (*Chanos chanos*) and mullet (*Mugil cephalus*) is 40-45%, 40% and 27-35%, respectively.

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.

Table 1. Nutrient requirements of different brackishwater fishes

Nutrient	*L. calcarifer*	*Mugil cephalus/ Liza tade*	*Etroplus suratensis*
Energy (Kcal/kg)	4000-4500	4000-4500	4000-4500
Protein %	45-55	27-35	30-32
Lipid %	6-18	6-9	6-8
Carbohydrate %	10-20	30-40	30-40

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. Carnivorous fish such as seabass can utilize lipids more effectively and lipid level as high as 20 % can be used in their diet. However, lipid level should be adjusted in diet considering the technological problems in feed manufacture and storage. Fish oil and soya oil are generally used as lipid source during feed formulation.

Fatty acids

Fish and shrimps 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.

Mineral requirement

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

Fish can absorb minerals directly from aquatic environment through gills and body surfaces or by drinking. Hence, dietary requirement of minerals is largely dependent on the mineral concentration of the aquatic environment. 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. In saline water, calcium (Ca) is abundant, which is absorbed by most aquatic animals. Since the availability of phosphorus (P) through water medium is poor, P should be made available through diet. Usually the preferred Ca:P ratio is 1:1 in feeds of aquatic species. Mono and dicalcium phosphate contain more available P than tricalcium phosphate. Incorporation of P should be very discrete in fish feeds, as most of it gets excreted leading to eutrophication. The dietary requirement of P ranges from 0.5-0.9% in fishes. The requirement of magnesium (Mg) in fish ranges between 0.04-0.3%. The requirement of zinc (Zn) ranges from 15-30 mg/kg diet for fishes. The requirement of iron (Fe) ranges from 150-200 mg/kg diet for fishes. Major deficiency symptoms of manganese (Mn) in fishes are cataracts and abnormal curvature of the backbone and malformation of tail. A dietary supplementation of 11-13 mg/kg restores normal growth in fishes.

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.

Vitamin requirement

Micronutrient such as vitamins and mineral significantly influence the growth and survival of fish and this cannot be synthesized by these organisms. Even though, some vitamin such as niacin can be synthesized by number of animals but are typically insufficient to meet physiological demand.

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

Table 3. 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

Genetic factors in broodstock management in finfish hatchery

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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.

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) Ammoniat

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)

Transportation methodology of brackishwater finfishes

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1. Introduction

There are two types of transport systems for live fish - the closed system and the open system. The closed system is a sealed container in which all the requirements for survival are self-contained. For example sealed plastic bag partly filled with water and oxygen. The open system consists of water-filled containers in which the requirements for survival are supplied continuously from outside sources. The simplest of these is a small tank with an aerator stone.

2. Factors associated with live fish transportation

Fish survival in a good state of health during transport is influenced by a number of factors, or combination of factors

2.1 Health of fish

The fish to be transported must be healthy and in good condition. Weakened individuals should be eliminated from the transportation. The fish to be transported, except for the larval stages should be starve for at least a day; if the fish is not starved, the possible time of transport is reduced to a half, though the conditions may be the same (Pecha, Berka and Kouril, 1983; Orlov et al., 1974). Natural ice is used to cool the water; the ice of carbonic acid should be avoided. As a guide ratio, 25 kg of ice will cool 1 000 litres of water by 2°C. If the water contains fish during the cooling process, the temperature drop should not be faster than 5°C per hour.

2.2 Dissolve oxygen

The most important single factor in transporting fish is providing sufficient level of dissolved oxygen. The ability of fish to use oxygen depends on their tolerance to stress, water temperature, pH, and concentrations of carbon dioxide and metabolic products such as ammonia. The crucial factors underlying oxygen consumption by fish in relation with oxygen metabolism during transport are fish weight and water temperature. Heavier fish and those transported in warmer water need more oxygen. During fish transport in closed systems with pressurized oxygen atmosphere, oxygen content in water usually is not a limiting factor because there is enough pressurized oxygen in a closed bag. In closed systems, slight shaking of the bag supports the penetration of atmospheric oxygen to water. During long steps when the bags with fish are left without movement, the fish may die though the oxygen reserve in the bag is still high.

2.3 pH, carbon dioxide and ammonia

The water pH level is a control factor because the proportions of toxic ammonia and CO2 contents are direct functions of Ph. With increasing transport time, CO2 production through fish respiration reduces water pH towards acidity. Water pH levels about 7–8 are considered as optimum. Rapid changes in pH stress fish, but buffers can be used to stabilize the water pH during fish transport. The organic buffer trishydroxylmethylaminomethane is quite effective in fresh and salt water. It is highly soluble, stable and easily applied. Dose of 1.3–2.6 g/litre are recommended for routine transport of fish (Piper et al., 1982). In general, for each milliliter of oxygen a fish consumes, it produces approximately 0.9 milliliters of CO2. Another important factor is chlorine concentration in water, although - like carbon dioxide - chlorine is also removed from the water by aeration. The concentration of 0.2 mg/litre is considered as dangerous (Shevchenko, 1978).

Ammonia (NH3) builds up in transport water due to protein metabolism of the fish and bacterial action on the waste. Decreasing metabolic rate of the fish by lowering the water temperature, and thus lessening fish activity, reduces the production of NH3. The production of NH3 by bacterial action can be decreased by starving the fish before transportation/ emptying the stomach and intestine. Temperature and time of last feeding are important factors regulating ammonia excretion. For example, trout held in water at 1°C excrete 66% less ammonia than those held in 11°C water. Fish larger than 10 cm should be starved at least 48 h; those 20 cm and larger should be starved 72 h (Piper et al., 1982). The amount of un-ionized ammonia increases as water temperatures and pH increase

2.4 Temperature

Water temperature is an important factor. When water temperature is low, the pH remains higher and fish metabolism decreases. The generally applicable zones of optimum temperatures for transported fish are 6–8°C for cold-water fishes and 10–12°C for warm-water fishes in summer. Naturally, these temperature ranges do not apply to the early stages of fish fry. The early fry of cyprinids cannot be transported at temperatures below 15°C.

2.5 Density

As to fry, the ratio of the volume of the fish transported and the transport water should not exceed 1:3. Heavier individuals, e.g., parent fish can be transported in a fish: water weight ratio of 1:2 to 1:3, but with smaller organisms this ratio decreases to 1:100 to 1:200 (Pecha, Berka and Kouril, 1983).

2.6 Stress during transportation

When fish were transported at higher densities, the levels of corticoids and glucose in the plasma increased and was retained when the transport was finished. During transportation besides transportation stress, stress may also be caused by the deterioration of water quality (Erikson *et al.,* 1997), salinity and temperature fluctuations (Mires and Shak, 1974), which might consequently alter the metabolism of the fish. Transportation of Grey mullet for stocking is mainly carried out in the fry and fingerling stages. Biochemical parameters serve as reliable indicators of physiological status of organism (Ferry-Grahm and Gibb, 2001). The tertiary response is the final stage, which leads to disease or exhaustion, growth retardation and finally death (Chatterjee et al., 2006). Catecholamine and cortisol induce glycogenolysis and gluconeogenesis respectively. Both processes together cause a rise in blood glucose level. Blood glucose and hepatic glycogen are therefore commonly measured parameters of stress response (Manush et al. 2005).

3. System of live fish transportation

3.1 Closed system

Transport of fry in polyethylene bags with oxygen is common example of closed system of transportation. It is generally used to transport fry.

3.2 Polythene bags

The bags used for fish transport in water with oxygen atmosphere are produced in a number of modifications. They are manufactured from a thin (soft) or thicker (hard) transparent polyethylene foil and usually have the shape of sack or sleeve. The bags of the traditional shape (sacs) usually have the dimensions of 0.8– 1.1 . 0.35–0.45 m. The upper end is usually fully open. The bottom either has a seam in the middle or consists of a rectangular piece of foil; the latter variant is better because it helps avoid losses of the fish squeezed in the corners. During transportthe bags with fry are placed in outer cases protecting the bags against mechanical damage. These cases can be cardboard boxes, suitable plastic

containers, wide polyethylene cans, and polystyrene boxes. If water with transported fry is to be cooled, bags with ice should be placed under the fish-transport bags on the bottom of the polystyrene box. It is not recommended to put the ice inside the transportbag. The volume of ice placed under the bag with transport water is usually 10–20% of the transport water. Other sealed container is generally made of cured plastics

3.3 Open system

In all cases of fish transport in open systems, it should be borne in mind that even a short-time transport of 10–30 m in open plastic or metal tanks should be done under the conditions of constant air or oxygen supply. In recent year most tanks are constructed are insulated, usually with styrofoam, fiberglass or urethane.

Styrofoam and urethane are preferred materials because of their superior insulating qualities and the minimal effect that moisture has on them. A well-insulated tank minimizes the need for elaborate temperature-control systems and small amounts of ice can be used to control the limited heat rises. Circulation is needed to maintain well-aerated water in all parts of the tank. Although most tanks presently in use are rectangular, the trend in recent years has been towards elliptical tanks, such as those used to transport milk. Self-priming pumps powered by gasoline engines are used to circulate water in many transport units. Pumping or aerating systems should be able to circulate at least 40 percent of the tank water per minute.

4. Mitigation of transportation stress

The chemical used during transportation to reduce the transportation and handling stress and increase the survival of the fish. Some of the commonly used chemicals include anaesthetics, water-hardening and oxygen-producing chemicals, bacteriostatics, buffering and antifoam.

4.1 Use of tranquilizer

It is best to sedate the fish in the holding facility for 30 min before loading and then to continue exposure to a lower concentration of sedative during transport. Anaesthesia usually applies only to transport brood fish. In practice, the fish are first tranquilized with the normal dose and put into the transport tank, where original concentration is diluted by 50 percent by adding the same amount of fresh water. The brood fish will remain tranquillized well in that diluted solution (Woynarowich and Horvath, 1980). As Woynarowich and Horvath (1980) assert, fish transport in cold water of 5–10°C is the simplest and best method of anaesthesia. Among the broad spectrum of anaesthetics, tricaine methanesulfonate (MS-222) and quinaldine (2–4 methylchinolin) appear to be used most frequently.

4.2 Use of sodium chloride and calcium chloride

Handling stress and delayed mortality of fish can be decreased by the addition of sodium chloride (NaCl) and calcium chloride (CaCl2) to the transport water. The sodium ion tends to “harden” the fish and reduce slime formation, and the calcium ion suppresses osmoregulatory and metabolic dysfunction. Calcium chloride may not be needed in hard water already containing high concentrations of calcium. Dupree and Huner (1984) recommended the addition of 0.1 to 0.3 percent salt and 50 mgL^{-1} calcium chloride. Some of the fishes that tolerate wide ranges of salt in the water, such as striped bass, tilapias, carp, can benefit from as much as 0.5 percent salt.

4.3 Oxygen source

Huilgol and Patil (1975) tested the use of hydrogen peroxide on transported carp fry and found that one drop (1 ml = 20 drops) of hydrogen peroxide (6 percentconcentration), applied to 1 litre of water,

increased the oxygen content by 1.5 mgL^{-1} when the temperature was 24°C. CO2 content and water pH were not influenced by the addition of hydrogen peroxide.

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