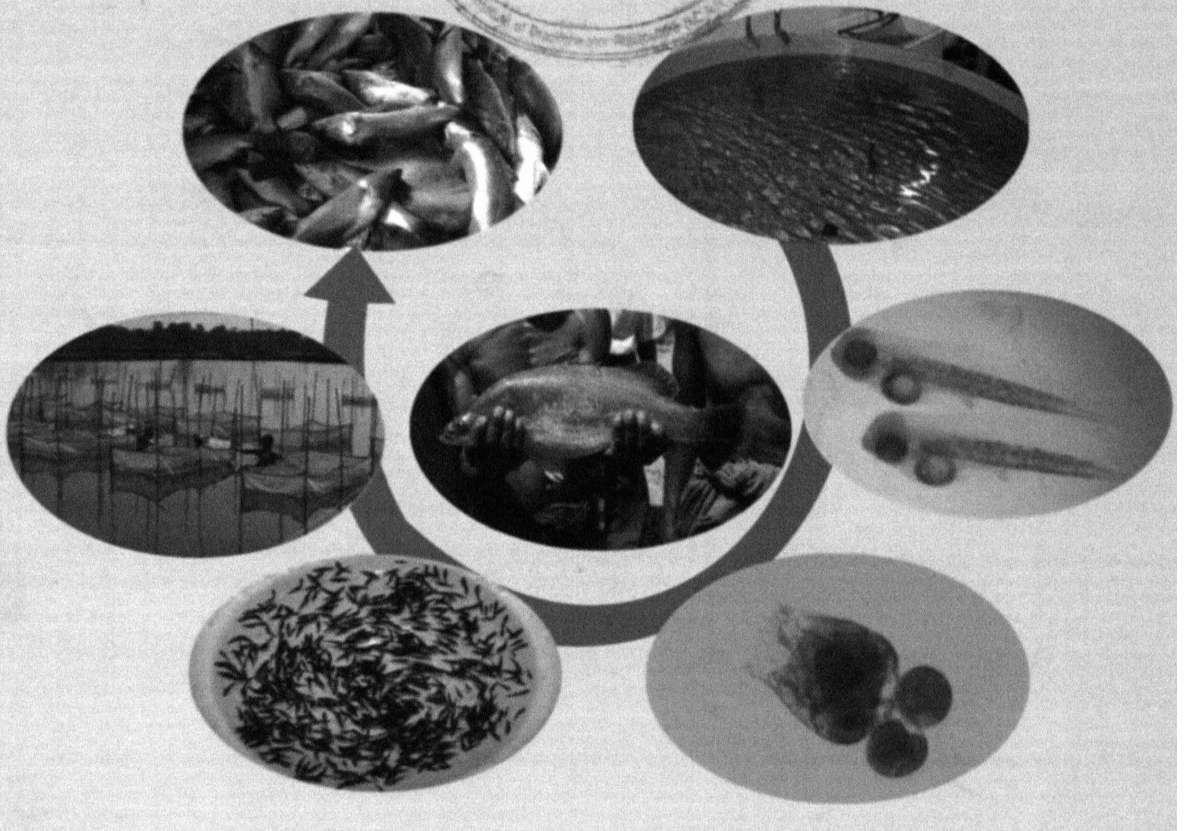
CIBA Special Publication No: 42

ASIAN SEABASS
FISH SEED PRODUCTION AND CULTURE



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NATIONAL FISHERIES DEVELOPMENT BOARD
HYDERABAD
September 2009

CENTRAL INSTITUTE OF BRACKISHWATER AQUACULTURE
(Indian Council of Agricultural Research)
75, Santhome High Road, R.A. Puram,
Chennai, Tamil Nadu - 600 028, India.

CIBA SPECIAL PUBLICATION No.42

Training Programme on

ASIAN SEABASS, FISH SEED PRODUCTION & CULTURE SPONSORED BY NATIONAL FISHERIES DEVELOPMENT BOARD, HYDERABAD

1-10th September 2009

Compiled and Edited by
Convener

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Dr. A. G. Ponniah
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FOREWORD

Brackishwater aquaculture has been under stress due to over dependence of single species the Tiger shrimp, *Peaneus monodon*. The need of the hour is diversification with a species that would fetch good price in the domestic market and has potential to be exported. Asian seabass (*Lates calcarifer)* known as Bhetki in parts of India is one of the fast growing sturdy fish species, capable of withstanding wide fluctuations in environmental conditions and can be farmed in pond, cages and pens in the brackishwater, freshwater and marine ecosystems. The culture of Asian seabass is in vogue traditionally based on stocking wild seed in coastal ponds wherever it is available, and allowing them to grow along with other fishes and shell fishes already in ponds and harvesting after 7-8 months duration. Through the technology developed by CIBA for controlled breeding and seed production and culture demonstration, Farmers have shown keen interest in taking up the sea bass farming. Interaction with farmers revealed more technocrats are required for propagation of seabass farming. CIBA has carried out on farm trials on nursery rearing and grow out of seabass involving entrepreneurs and farmers. Monoculture demonstration with support of NFDB is underway in three different agroclimatic regions in India.

In the above context, a ten days sponsored training course on Asian Seabass fish seed production and culture by NFDB, Hyderabad is being organized at this institute during 1-10th September 2009. This training programme is appropriate endeavour to fulfill the requirement of trained manpower in the above areas of fisheries in our country. I do believe that participants who have come from different state will make best use of this effort of CIBA and shall be able to extend and apply there experiences in development of seabass farming in respective states. I express my compliments to my colleagues in organizing the training programme and I am sure that, this publication would be useful as study material for the participants in understanding the seed production and culture technology.

A.G. Ponniah

Chennai
1st September 2009

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PREFACE

Brackishwater Aquaculture is considered as one of the potential growth sectors in India owing to the vast resources. Phenomenal growth witnessed during 1980's and early 1990's was synonymised with farming of Tiger shrimp, *Penaeus monodon*. The outbreak of uncontrollable viral disease like the WSSV coupled with other social, environmental and economical issues have forced the farmers to look for an alternative group of aquatic organism for the sustainability of Brackishwater aquaculture. In this context, Asian seabass, *Lates calacrifer* know as Bhetki in many parts of India and a highly relished table fish assumes greater significance. Efforts are being made to poularise farming of this value added fish. Quality seed availability in adequate quantity is an important factor in the development of large scale farming of Asian seabass. The technology for controlled breeding and seed production was achieved at Central Institute of Brackishwater Aquaculture (CIBA), Chennai for the first time in India during 1997 and since then the technology has been refined, improved and has evolved as commercial technology and today year round seed production is possible. Annual trainings are given to perspective entrepreneurs, farmers and technocrats in the seed production and culture of Asian seabass at CIBA. Considering the aquaculture potential of Asian seabass, farming and to create confidence amongst farmers, entrepreneurs and officers of State Departments, this training programme is organized with the support of National Fisheries Development Board, Hyderabad for 10 days from 1-10th September 2009.

The special publication of this training programme is a compilation of information on the seed production and culture of Asian seabass and other related aspects important for the development of aquaculture. I am overwhelmed by the spontaneous response received from various stakeholders for participating in the programme. The involvement of my colleagues, for contribution of articles in the field of specialization is greatly acknowledged.

We are extremely thankful to Dr. P. Krishaniah, Chief Executive and officials of National Fisheries Development Board, Hyderabad for taking interest in the training programme and sponsoring the same with financial assistance.

Dr.A.G. Ponniah, Director, CIBA, took keen interest, offered valuable guidance and suggestions for the conduct of this training programme. My colleagues, Dr.M. Kailasam and Dr. J.K. Sundaray Co-conveners of this programme have helped immensely in organizing and conduct of this programme. The support and assistance from the technical officers and other staff from the Fish Culture Division and from Administration & Finance for the training programme is gratefully acknowledged. I hope this manual would serve as study material in providing valuable information on seabass seed production and culture as well in all related aspects important for Brackishwater aquaculture development.

A.R.T.Arasu
Course Convener

Chennai
1st September 2009.

CONTENTS

PROTOCOLS & PROCEDURES FOR CONTROLLED BREEDING OF ASIAN SEABASS *LATES CALCARIFER*

A.R.Thirunavukkarasu, M.Kailasam and J.K. Sundaray

Seabass (*Lates calcarifer)* spends most of its growing phase in confined waters in the coastal and inland areas and migrates to sea for maturation and spawning. Sea is an environment with its characteristic feature of high salinity <30 ppt, pressure, pH <8, low temperature and low intensity of light. The environment conditions prevailing in the sea may be essential for activating the hormones responsible for reproduction. Matured fish can be made to spawn under captive condition, if the sea-conditions can be simulated. But simulating such conditions in hatcheries has limitations and it is not possible in all the places. However by administering extraneous hormones responsible for ovulation and spawning will be helpful to induce spawning. Many hormones like, HCG, ovaprim, ovatide, carp pituitary, salmon pituitary etc. are used but in case of seabass breeding., Leuteinizing Hormone and Releasing Hormones analog (LHRHa) is found to be more effective.

1. Induced Spawning

Spawning is a "process of release of sexual gametes". Since sexes are separate in the fish, both male and female matured fishes have to be selected for spawning. The fertilization is external.

1.1 Selection of spawners

Matured female fishes will have ova with diameter more than 450 μ. Males will ooze milt if the abdomen is gently pressed. The gonadal condition is assessed by ovarian biopsy (Refer – Induced Maturation). Brood fishes selected for induction of spawning should be active, free from disease, wounds or injuries. Female fishes will be around 4 – 7 kgs and males will be 2.0 – 3.0 kgs. Since sea bass spawning is found to have lunar periodicity, days of new moon or full moon or one or two days prior or after these days are preferred for inducing the spawning.

1.2 Induced Spawning by Hormone Injection

The commonly used hormones in the finfish hatcheries for induced spawning are:

LHRH-a - Luteinizing Hormone Releasing Hormone analogue (Available with SIGMA CHEMICALS – USA – ARGENT CHEMICALS)

HCG - Human Chorionic Gonadotropins. (Available in Pharmacy – medical shops)

Ovaprim - A Glaxo Product

Puberogen -

Carp pituitary glands -

Pimozide -

But in the case of seabass LHRH-a hormone is found to be effective with assured result though other hormones can also be used singly or in combination.

2.1 Hormone dose

After selecting the gravid fishes the requirement of hormone to be injected is assessed.

The dosage level has been standardized as LHRHa @ 60 – 70 μg/kg body weight for females and 30 – 35 μg/kg body weight for males. The hormone in the vial (normally 1 mg) is dissolved in distilled water of known volume (5 ml). Care should be taken that hormone is thoroughly dissolved. The weight of the brood fishes is assessed and the required hormone is taken from the vials using a syringe.

For eg:

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 x 6 = 420 μg for female fish

go from the vial $\frac{1}{200}$ x 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 x 3 = 105 μg.

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

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

Hormone Injection

The fish is held firmly. To reduce the activity, the snout portion is covered with a hood. After removing one or two scale just below the dorsal fin – above the pectoral region the syringe needle is inserted into the muscular region and the hormone is administered intramuscularly gently.

Time of Injection

Since the spawning normally occurs in the late evening hours, when the temperature is cool, hormone is injected normally in the early hours of the day between 0700 – 0800 hours.

Spawning Tanks

Spawning tanks size depends upon the size of the fish selected. Normally 10 – 20 tonne capacity tanks with provision for water inlet, drainage, overflow provision and aeration is used.

Sex Ratio

Female seabass are generally larger (more than 4 kg.) and the males are smaller (in the size of 2.0 – 3.0 kg). To ensure proper fertilization normally two males are introduced for one female in the spawning tank.

Spawning

Fishes injected with LHRH-a hormone response for spawning after 30 – 36 hours of injection. Prior to spawning gradual swelling of the abdomen will be seen indicating the ovulation process. Spawning normally occurs late in the evening hours 1900 – 2000 hours. At the time of spawning the fishes will be moving very fast and in the water surface a milky white substance will be seen. There will be a fishy odour which can be felt few meters away. Prior to spawning activity the males and the female will be moving together exhibiting courtship.

Spawning activity in seabass coincides with lunar periodicity. During full moon or new moon days, the activity is found to be in peak. Hence, induced spawning is done during new moon/full moon or one or two days prior or after these days. Seabass has high fecundity. It is a protracted intermittent spawner (releasing eggs batch by batch). In one spawning the fish may release 1.0 – 3.0 million eggs. The process of spawning will follow during subsequent day also. If the condition is good, both female and male respond simultaneously resulting spontaneous natural spawning and fertilization is effected.

Fertilization

Fertilization is external. In natural spawning of seabass in good maturity condition, fertilization will be 70 – 90%. The size of the fertilized eggs will be around

0.75 – 0.80 mm. The fertilized eggs will be floating on the surface and will be transparent. The unfertilized eggs will be opaque and slowly sink to bottom. Due to water hardening sometime, even the unfertilized eggs, for short duration will be on the sub-surface but will sink subsequently.

7. Egg Collection

The fertilized eggs can be collected by any one of the following methods.

7.1 Overflow method

After spawning and fertilization, the water level in the spawning tanks can be increased and allowed to overflow through overflow outlet. The eggs will be pushed by the water flow. Below the overflow pipe a trough covered with bolting cloth of mesh size 150 – 200 u is kept. The water with the egg is allowed to pass through. The eggs are collected in the next bolting cloth washed and transferred to the incubation tanks.

7.2 Scooping/Seine net collection method

Since fertilized eggs will be floating on the surface, a bolting net cloth of 150 – 200 u mesh size can be used for collecting the eggs from the surface. The cloth is stretched as net and towed along the water surface. The collected eggs after washing are transferred to the incubation tanks.

7.3 Siphoning method

The water in the spawning tank is siphoned into small tank covering with collection net cloth through which the water will be allowed to pass through. The eggs collected in the net cloth are transferred periodically to incubation tanks.

8. Incubation and Hatching

The eggs collected from the spawning tank are washed to remove the debris that would have adhered to and transferred to the hatching tanks for incubation and hatching.

The hatching incubation tanks can be 200 – 250 litres capacity cylindro-conical tanks. Eggs are kept @ 100 - 200 nos./litre density. Continuous aeration is provided. Temperature of 27 – 28°C is desirable. 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

Hatched larval size : 1.4 to 1.5 mm

After hatching the larvae are transferred to larval rearing tanks. The unhatched/unfertilized eggs (dead eggs) in the incubation tank can be removed by siphoning. The larvae are scooped gently using scoop net and transferred into buckets of known volume. After taking random sample counting depending upon the number required to be kept in the rearing tanks, larvae will be transferred to rearing tanks.

FINFISH BREEDING TECHNIQUES

A.R.Thirunavukkarasu, M.Kailasam and J.K. Sundaray

2.1 ## 1. Introduction

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

2.2 ## 2. Selection of breeders

Breeders can be obtained either from wild or from broodstock developed in captivity. One of the problems faced in induced breeding is that variations occur in the gonadal development among individual fish both in the wild and in broodstock developed in captivity. Successful induced breeding depends upon the selection of the recipient fish at the proper stage of the gonad development. Normally, the external characters like fullness of belly, colour and state of swelling of genital opening such as protruding pinkish/reddish, genital papilla, softness and 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 nonadhesive. The average ova diameter has to be determined and this is

used as an important criterion in the selection of females for induced spawning. In the case of males, maturity is ascertained by applying pressure on either side of the belly. In the case of fish in mature condition, milt will be flowing through the genital opening on application of gentle pressure.

3 Sex determination

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

4. Methods of Breeding

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

4.1 Artificial fertilization by striping

In this method spawners are obtained from wild during the natural breeding season. In seabass breeding is related to lunar cycle. Again breeding occurs before midnight during high tide. Eventhough 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 wil be around 0.7 to 0.8 mm with large oil globule. The eggs will be almost transparent. The ripe eggs will be scattering individually whereas unripe eggs

tend to group together when put them 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 striped 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 so that all eggs are just kept floating and mixed well for 2 – 3 minutes. Then the eggs are washed 3 to 4 times using a strain to remove all mucus and other tissues. Thereafter the fertilized eggs are distributed to incubation tanks.

4.2 Environmental manipulation

This technique is usually followed in broodstock developed in captivity. About a month prior to the spawning season the mature females and males are 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 allow the water temperature to go up to above 30^{0}C. By dusk fresh sea water is added to the spawning tank to simulate the raising tide conditions and simultaneously water temperature also declines to around 27^{0}C. The fish that is in right stage and good condition will spawn in the same night or during the second 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.

4.3 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 Luteinzing hormone – releasing hormone alalogue (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 maturity of females are examined by taking out a sample of the eggs using a polyethylene canula 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 to 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 canula 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 canula for a distance of 3-4 cm from the cloaca. The other end of the canula is held in the mouth of the operator and the eggs are aspirated into the tube by the operator. When the eggs enters the cannea, the cannea 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 days. 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.

BIOLOGY OF SEABASS *LATES CALCARIFER*

M.Kailasam, and A.R.Thirunavukkarasu

Asian Sea bass (*Lates calcarifer*) known as Bhetki in many parts of India is an important food fish. Though it forms negligible in perch fishery in commercial catches, it is highly suitable for farming. Sea bass is an euryhaline species capable of withstanding wide salinity fluctuations. It is a catadromous fish migrating towards sea for breeding. Seabass is widely distributed in the tropical Indo-pacific region. It is a fast growing fish and suitable for culture both in cestrum ponds and in cage system.

Taxonomy

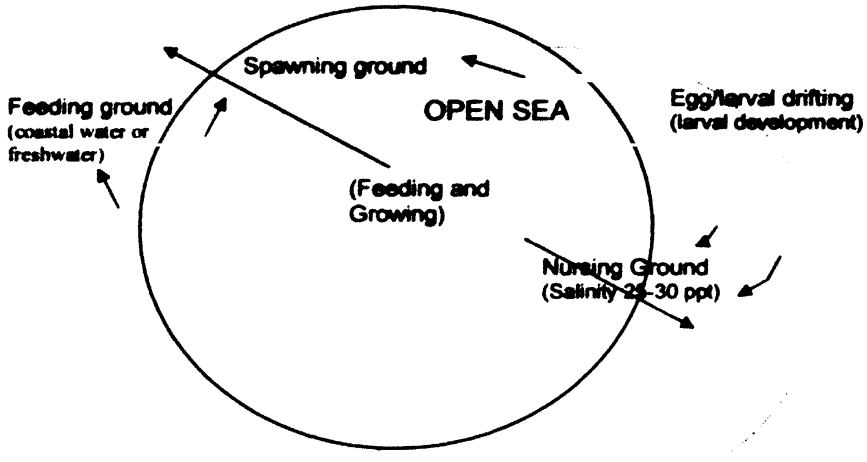
Phylum	-	Chordata
Sub-phylum	-	Vertebrata
Class	-	Pisces
Sub-class	-	Teleostei
Order	-	Perciformes
Family	-	Centropomidae
Genus	-	*Lates*
Species	-	*Lates calcarifer* (Bloch)

Vernacular names

Tamil	- Koduva
Malayalam	- Kaalanji
	Narimeen
Telugu	- Pandu kappa
Marathi	- Jitada
Bengali	- Bhetki

Life History

Seabass though spends the growing phase in shallow water bodies like estuaries, backwaters and freshwater, 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. Post larvae transported passively through tidal currents and enter into coastal swamps and fresh water bodies. They stay in this productive and safer environment and attain rapid growth. Seabass spends most of its growing period under these conditions. Adult fish migrate towards the mouth of the river from adjacent areas into the sea belt when salinity is high which favour sexual maturation and spawning.



Migration Pattern of *Lates calcarifer* (BLOCH)

Food and feeding habits

Seabass is carnivorous in nature. However, juveniles are omnivores. Seabass is an opportunistic predator, whose diet changes at different ages of various size group. It feeds mainly on zooplankton in early stages and as they grow changes to feeding on young fishes and shrimps. They show preference for pelagic fish rather than benthic crustaceans as the prey is large. However, juvenile seabass even consume smaller sizes of seabass of the same age group as whole and can cause reduction in the survival rate.

BREEDING BIOLOGY

In seabass, sexes are separate. But it is difficult to distinguish externally. However, the following differences could be made. Seabass of the same age group, males are generally smaller (2-3 kg) and with more slender and narrower body depth than females. During the spawning season, the milt can be observed at the genital opening if slight pressure is applied on the abdomen of the mature male. The female (more than 3 kg) can be recognized from the big soft round belly with the red-pink papilla extruding out at the urogenital aperture. If the female has a fully ripe egg, the egg will be visible when the abdomen is strongly pressured by hand. Mature females can be examined through biopsy also.

Fecundity

Fecundity of seabass is related to the size and weight of the fish. The fecundity of seabass varied between 1.0 and 20.0 million eggs.

3.5 Sexual maturity

In the early life stages (2.0 – 3.5 kg body weight) majority of the seabass are males but when they attain the body weight of 4 kg and above, majority become females. Seabass is sexually protoandrous hermaphrodite fish. Even though, many literature states that Seabass mature at the age of three, maturation of captive stock was noticed even at the age of two.

3.6 Female

Appearance of follicle tissues in ovary is the first stage for development of gonadal tissues in females. As, gonad develop, the gonadopore or genital opening become visible just below the anal pore. Gonad development is extremely rapid, ovulation of eggs leads to belly enlargement just before spawning in female. The gonads of seabass are strongly dimorphic and size of gonad varies on different size groups. Usually, the oocytes in the posterior end of the ovary are larger in size than oocytes of anterior region indicating that the process of ovarian development is a continuous one and spawning process is multiple.

Seabass cultured in low saline pond water and can mature after transferring them in to seawater with 30 ppt salinity. Salinity is a key factor, essentially required for maturation.

3.7 Male

Males attain maturity at the size of 25 cm total length. When the fish are 3-5 years old (56-65 cm in total length) fully mature males can be recognized. The testes of male seabass were first become distinguishable macroscopically by the appearance of furrows in their ventral surface at which time they are semi-transparent, strap or breed like, very thin, and often bordered by fat.

3.8 Sex inversion

Male participate in spawning several times before changing into females. Male turn into female generally when they are 65-85 cm in total length and about 5.0 kg weight in India and Thailand. However, in Australia, the male become females when they are 82-100 cm in length and about 7.0 kg weight. In a fully mature female, the diameter of oocyte usually ranged from 450-530 µm.

3.9 Spawning

In India spawning season noticed during July to November. Spawning usually takes place in the sea. Seabass is multiple spawner and release the eggs batch by batch continuously upto three days. Fertilized eggs are usually transparent and pelagic form and it can be easily drifted into coastal waters for larval development. Maturation process initiates during on set of summer and continued upto October. Spawning takes place during May-November. Restoration of gonad takes place during on set of north east monsoon in the east coast.

3.12 Growth

Asian seabass is a fast growing fish. Normally in the first year itself it would attain 0.8 – 1.0 kg and in the second year it attains size upto 2.0 kg. Large size seabass even upto 6.0 kgs are caught from the estuaries and in shore waters by artisanal fisherman mainly using hook and line. The growth of seabass depends upon the density, the abundance of the feed in the environment etc. However, under culture condition, wide variations have been observed in the growth of seabass. Even though same size group seed due to the natural differential growth is in the first year it self, fishes from 0.40 kg to 4.0 kg were harvest indicating potential of seabass growth.

Table 1 : Stages of gonadal development in Seabass *Lates calcarifer*

Stage	Female	Gonadal condition male
I Virgin	Glassy, rounded and ¼ the body cavity in lengh	Colourless thin strap lying along the blood vessel. One half body cavity in length
II Maturing virgin and recovering spent	Definite gonadal appearances the same length as stage – I	Whitish and has assumed a definite gonadal appearances. The same length as stage - I
III Developing	Fills half the body. Eggs can be distinguished separately.	Fills half the body cavity. Whitish
IV Developed gonad	Yellowish and easily detectable as female. Ovary about 2/3 of body cavity	Whitish with gonadal appearance
V Fully ripe	Eggs are separate and fill the entire body cavity	Milt fills the body cavity and can be expelled without difficulty, white and sticky
VI Spent	Ovary flaccid. May have some eggs remaining.	Testes thin although not as flaccid as female. Some spawner may have the testes remain and fill to one half of body cavity.
VII Resting	Ovaries reddish and small, easily confused with stage - II. Identification under microscope may be necessary	Testes are small and thin, they are sharp thin, they are sharp viewed from the edge.

4 HORMONAL CONTROL OF REPRODUCTION IN FISHES

J.K.Sundaray, A.R.T. Arasu and M. Kailasam

Introduction

Reproduction in fish, as in other vertebrates is regulated by hormones from brain, pituitary and gonads. Hormones originating in sources other than these may help in some cases but their influence is not obligatory. In general reproduction in fish is al, starting from the recrudescence of gonad to final maturation usually occurs in particular time of a year. Endocrine cycle therefore closely corresponds to the seasonal gonadal cycle. Two environmental factors, temperature and photoperiod, plays a major role in the release and function of hormones.

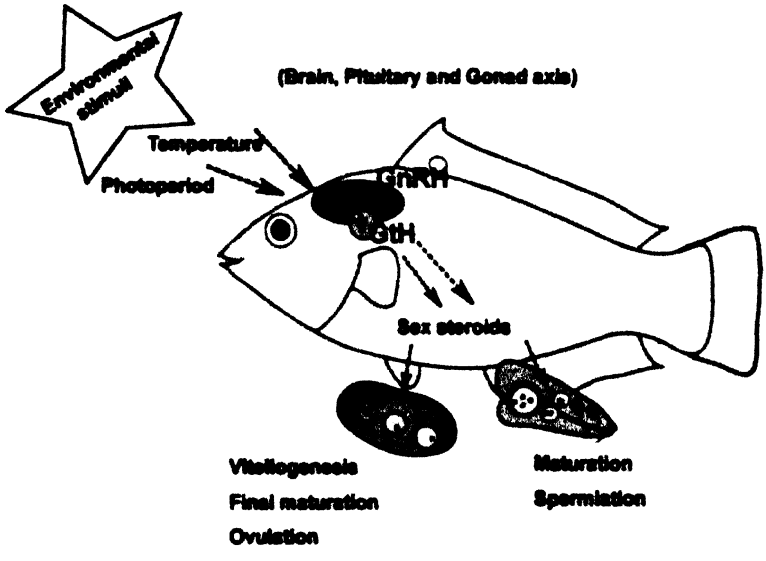


Fig. 1. Schematic representation of hormonal control of reproduction in fishes

It is now known that these environmental cues mediate their effect via release of GnRH (gonadotrophin releasing hormone), which reaches the pituitary through portal circulation where it binds gonadotrph cell membrane receptor and cause GtH (gonadotrophin) release into circulation. GtH then travels through the circulation and reaches ovary or testis where it occupies specific receptor in the cell membrane of theca and granulose cells of ovary or leydig cell of testis resulting stimulation of steroid hormones synthesis in these cells. Various kinds of steroid hormones from gonads induce development, growth and final maturation of germ cells and their release to the exterior aquatic medium effect the process of fertilization.

Endocrine regulation of spermatogenesis, spermiation and sperm maturation

Spermatogenesis is an extended process of differentiation and maturation of germ cells resulting in haploid spermatozoa. The principal stimuli for vertebrate spermatogenesis are thought to be pituitary gonadotropins and androgens. The mechanisms of action of these hormones remain unresolved. In cultivated Japanese eel, type A and early type B spermatogonia are the only germ cells present in the testis. They are primitive spermatogonia that have not begun to proliferate. Sertoli and Leydig cells are also poorly developed. A single injection of human chorionic gonadotropin (HCG) induces development of mature sperm within 18 days. One day after an HCG injection, Leyding and Sertoli cells become markedly active. Three days after treatment, spermatogonia begin mitotic proliferation and late type B spermatogonia appear. Following approximately 10 mitotic divisions, spermatogonia then begin meiosis. Spermatocytes with synaptonemal complexes first appear in testes 12 days after HCG injection. Spermatids and spermatozoa are observed within 18 days.

Culture of testicular fragments in media containing HCG induced the entire process of spermatogenesis from premitotic spermatogonia to spermatozoa within 24 days (Miura et al., 1991a). This HCG induced spermatogenesis was accompanied by a marked activation of Sertoli and Leydig cells which occurred prior to the onset of spermatogonial proliferation. Furthermore, addition of HCG to culture media containing testicular fragments induced a marked, rapid increase in 11 ketotestosterone (11-KT), but not testosterone, production. The enzymes (11β-hydroxylase, 11β-hydroxysteroid dehydrogenase which convert testosterone to 11-KT) responsible for synthesis of 11-KT have been cloned and sequenced in many fishes and the Northern blotting analysis revealed that neither 11β-hydroxylase nor 11β-HSD mRNA transcripts were present in testes before HCG treatment but both were abundant in testes 1 day after HCG treatment. It has been evident that 11-KT can induce all stages of spermatogenesis *in vitro* within 21 days (Miura et al., 1991b). Taken together, these results suggest hormonal induction of spermatogenesis in eel testes involves gonadotropin stimulation of Leyding cells to produce 11-KT which then activates Sertoli cells leading to the completion of spermatogenesis.

A distinct shift in the steroidogenic pathway from 11-KT to 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-DP) appears to occur in the testes of several species of teleost fish around the onset of spermiation. These result suggest that 17α, 20β-DP plays a role in the process of final testicular or sperm maturation in some teleost fish. Using different testicular preparations, we showed that the site of 17α, 20β-DP production is sperm, but its production depends on the provision of a precursor steroid (17α-hydroxyprogesterone) which is produced by testicular somatic cells under the influence of gonadotropin.

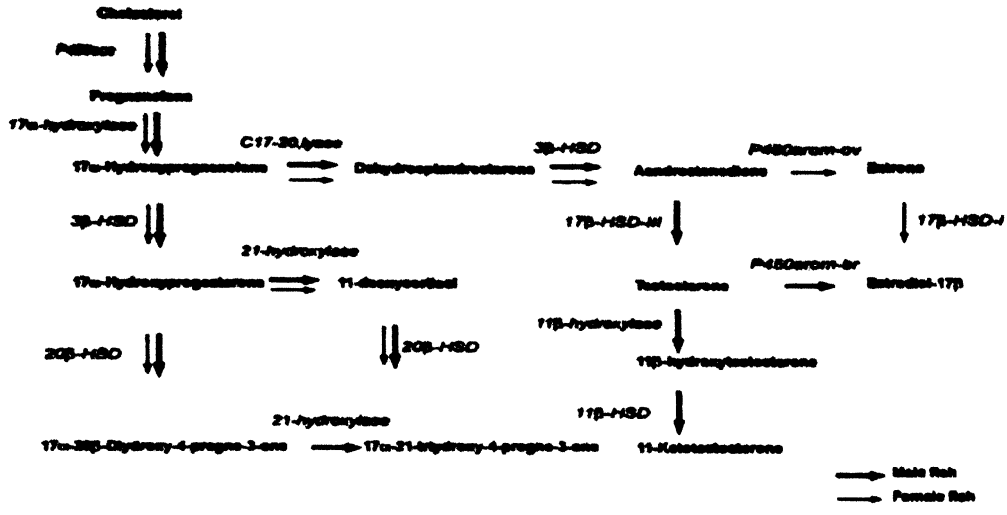


Fig.2. Schematic representation of gonadal steroid biosynthesis in fish

Spermiation, which is a prerequisite step for successful fertilization, generally occurs in teleosts immediately before or during spawning period. A single injection of salmon gonadotropin to non-spermiating salmon induced precocious spermiation 1-2 months prior to the normal spermiation period, concomitant with a marked increase in plasma levels of 17α, 20β-DP. Two successive injections of 17α, 20β-DPcaused precocious spermiation. Neither testosterone nor 11-KT was effective. These results provide evidence to suggest that 17α, 20β-DP is a testicular steroidal mediator of gonadotropin-induced spermiation in salmonid fishes.

Endocrine regulation of vitellogenesis and ova maturation

Oocytes of teleost fishes, like those of other nonmammalian vertebrates, grow while arrested in the first meiotic prophase. The principal event responsible for the enormous growth of fish oocytes, vitellogenesis, involves the sequestration and packaging of a hepatically derived plasma precursor, vitellogenin, into yolk protein. The period of oocyte growth is followed by maturation which occurs prior to ovulation and is a prerequisite for successful fertilization. Oocyte maturation is defined as the reinitiation and completion of the first meiotic division and subsequent progression to metaphase II.

Two gonadotropins, FSH-like GTH-I and LH-like GTH-II, have been characterized from several species of teleost fish by either protein purification or subunit cDNA cloning. GTH-I appears to be involved in the process of vitellogenesis/spermatogenesis whereas GTH-II is responsible for oocyte maturation and ovulation/spermiation. The ability of gonadotropins to modulate gonadal function depends not only on circulating levels of gonadotropins, but also on the expression of the appropriate receptors in the gonad.

Estradiol-17β and 17α, 20β-DP are now known to be important for oocyte growth (estradiol-17β) and maturation (17α, 20β-DP, maturation-inducing hormone)

(Nagahama and Adachi, 1985). Production of both steroid hormones follows a classic two cell type model in which the precursor steroids produced in the thecal layer are converted to estradiol-17β and 17α, 20β-DP, respectively, in the granulosa layer (Kagawa et al., 1982). Estradiol-17β is introduced the hepatic synthesis and secretion of vitellogenin. A dramatic switch in the steroidogenic pathway from estradiol-17β to 17α, 20β-DP production occurs in ovarian follicle cells immediately prior to oocyte maturation. This switch, a prerequisite for the growing oocyte to enter the maturation phase, requires a complex, integrated network of gene regulation involving cell-specificity, hormonal regulation, and developmental patterning. To know the detail network of steroid biosynthesis in the gonads steroidogenic enzymes have been isolated and characterization of the genes encoding the steroidogenic enzymes responsible for estradiol-17β and 17α,20β-P biosynthesis have been completed in several fin fishes.

In rainbow trout, mRNA levels of P450scc, 3β-HSD, and P450c17 are barely detectable in ovarian follicles during the midvitellogenic stage, but become abundant in postvitellogenic and maturing follicles. In contrast to several other teleost species, only one P450arom transcript and protein have been isolated from tilapia (Chang et al., 1997). Both P450arom mRNA and protein levels are low in early vitellogenic follicles, increase in midvitellogenic follicles, and decline to non-detectable levels in postvitellogenic follicles. Changes in follicular conversions of exogenous testosterone to estrogens (i.e. aromatase activity) correlate with P450arom mRNA and protein levels indicating that estradiol-17β synthesis in closely related to P450arom expression within follicles. cDNA clones encoding 20β-hydroxysteroid dehydrogenase (20β-HSD) a vital enzyme for conversion of 17α-hydroxyprogesterone to 17,20β-P have been isolated from many fishes and its expression has been observed during oocyte maturation stage.

Recent investigations of hormonal control of teleost gametogenesis have provided significant insight into the mechanisms controlling gametogenesis while simultaneously identifying crucial areas of future research in the area of gonadotropin control of gametogensis.

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GENETICS AND FISH STOCK IMPROVEMENT

T.C.Santiago, K.K.Vijayan ,S.V.Alavandi and N. Kalaimani

In plants and animals, application of genetic principles such as selection, hybridization etc had contributed significantly in increasing production capability, survival rate, growth and in quality improvement of the product. But in aquatic species, the application of genetics is in infant stage and has been restricted to areas like ploidy manipulation sex manipulations etc, owing to several difficulties. Aquatic animals still offer a great potential for improvement through genetic manipulations because of the qualities viz. external fertilization high fecundity etc.

For the stock improvement programmes of fishes, there can be two approaches, which on their own or in combination , can bring about huge economic gains in aquaculture. One is the traditional quantitative genetic approach and the other is the molecular genetic approach of relatively recent origin.

Quantitative genetics:

The basic tool of the breeder in the quantitative genetic approach is the selection of brood fishes based on the relative genetic merit. The expected genetic gain through selection depends upon the heritability, additive genetic variance and intensity of selection. So for the effective implementation of any selection scheme in a breeding programme, there is a need to estimate the genetic parameters like heritability of the traits under selection and genetic correlations among the traits under selection. Asian Sea bass, *Lates calcarifer* offers a good scope for improvement through selection, because of the huge variability in growth rate within the same population. The preliminary studies on the genetic parameters showed a positive trend and medium to high variability for body weight at early stages of life.

Hybridization:

In any genetic/stock improvement program it is necessary to breed the candidate species artificially under controlled conditions. The success of induced breeding of paved the way for research on genetic improvement of fishes. Simple interspecific and intrageneric hybridizations were done to produce and evaluate the fishes with useful traits for aquaculture. Hybridization is one of the methods used for combining desirable traits of selected species with experiments demonstrating a high

level of compatibility among fishes. Fishes with distinct morphological characteristics are suitable for hybridizations. Mature interspecific and intergeneric hybrids could be induced to produce F1 progeny or backcross and triple cross hybrids. Growth exhibited by most hybrids are intermediate, i.e. better than the slow growing parent as in the case of interspecific hybrid crosses . However, it is necessary to prevent indiscriminate hybridization.

Chromosome manipulation:

Chromosome manipulation as a tool to improve genetic status of fish under captive conditions. Success was achieved in carps. Similarly it is possible to achieve such success in sea bass is also possible. The sperms were irradiated so as to destroy the male genetic material and theses sperms were used to fertilize the eggs and induce the development of the egg. Using temperature shock or chemical treatment or pressure the development of the cell is disrupted and thus 2n number of chromosome is restored. Such eggs are allowed to develop and produce progeny having the genetic material for the female only. Similarly, androgenic animals can also be produced. Here the genetic material of the eggs are destroyed by UV. irradiation or some similar methods. The irradiated eggs are allowed to be fertilized by intact sperms and thus the genetic material comes from the male. As in the gynogenesis , the cell wall formation is arrested by the physical means and thus diploid condition is restored. Triploidy is another condition that can be induced in fishes. Tilapia is an example of this . The triploid individuals show better growth since they are sterile. This approach helps in unwanted reproduction in grow out ponds. Current research in chromosomal manipulation in fisheries is minimal. However, it offers better future in aquaculture.

Genetic characterization:

Identification and genetic characterization of wild and hatchery populations are important since broodstock with good genetic background is necessary for a successful breeding program. The knowledge from such investigations can be used in optimizing and sustaining yield, stock management and conservation of genetic diversity. Fishes can be easily characterized at molecular levels using modern techniques such as RFLP, RAPD. These approaches require specific restriction enzymes and primers. Such works are initiated in CIBA to characterize sea bass.

Gene mapping:

Gene mapping is done for localization and functional characterization of economically important trait genes that will improve the breeding programme through marker assisted selection. The work involves isolation and development of highly polymorphic type DNA markers and subsequently development of a genetic linkage map for the fishes. The long term objective is to identify trait associated genes such as disease resistance and body growth. DNA finger printing can be used in this approach to determine DNA profiles of selected unselected and control stocks used in selective breeding programme. This techniques can be used as genetic tagging for breeding programmes.

Gene banking:

This is one of the techniques to conserve the endangered species. However it is used to conserve the germplasm of the desired species. Construction of gene library is one of the means to store all the genetic material form which genes of interest can be isolated for future use. Similarly, cDNA libraries can be also constructed to isolate the genes that are expressed by the animals. Apart from this the milt of the fish can be also used to be used later stage for breeding programs. High quality male sperms can also be used for cryopreservation that can be of great help in producing high quality fries.

Transgenic fish:

Transgenic fish is defined as a fish where foreign genes are introduced. This modern technique is used in plants and higher animals. The same technique has also been used in fishes with success. Though it is a sophisticated approach it is possible to use this technique in fishes. Various laboratories have produced transgenic fishes. Using these techniques it is possible to develop fishes with desired economic characteristics such as taste, feed conversion efficiency, growth and disease resistance. The success of introducing the genes of interest into the animal so that it is passed on to the offspring is only about 2 percent it is worth the attempt owing to its economic importance.

Future of genetic research:

The following are the key areas for genetic research in the future.

- Development of breeding programmes for important aquaculture species

- Selection of important traits such as disease resistance and growth for selection studies.
- Application of molecular markers and genetic techniques for breeding programmes
- Research on genetic engineering
- Testing and dissemination of research outputs to end users.

CRYOPRESERVATION OF SPERMATOZOA OF ASIAN SEA BASS *Lates Calcarifer*

Shiranee Pereira

INTRODUCTION

Cryopreservation is the branch of biology that deals with the preservation of living tissues /gametes/embryos in ultra cold temperature (-196 ° C). At this temperature, the metabolic activities of the cells are arrested but they remain viable. Their normal functions can be reactivated after proper thawing. The advantages and uses of cryopreservation of spermatozoa in fish breeding are:

1. For the short term storage of milt in hatcheries to overcome problems like asynchronization in maturation and for the selective use of spawners.

2. Shipping of spermatozoa. Milt can be cryopreserved and transferred to other places for propagation through artificial methods.

3. In genetic studies:

   a) To cope with the genetic problems like in breeding associated with small populations. Cryopreserved milt collected from many males in previous generations can be used to improve the effective population size. The cryopreserved milt guarantees at least 50% of genetic exchange thereby avoiding the deleterious effects of in breeding.

   b) For the production of intra specific and inter specific hybrids.

   c) Makes it possible to store milt for an indefinite period.

   d) Ex situ conservation of endangered species is possible through cryo preservation.

THE PRINCIPLE AND PROCESS OF CRYOPRESERVATION

Cryo preservation is based on the principle of vitrification and involves three basic steps: freezing, storage and thawing. During normal freezing (that is without the addition of a cryoprotectant) several physico- chemical changes take place within the cell and its surrounding area. The plasma membrane that surrounds the cell gets affected by cold shock. Plasma membrane consists of lipid protein bi layer and controls the transport of metabolites and ions. Lipid remains in liquid state at normal temperature. At low temperature lipids gets frozen which affects the permeability and structural integrity of the membrane. Initially ice crystal formation occurs in the extra cellular medium due to freezing. As a result, the extra cellular medium becomes hypertonic to the cell. To maintain the osmotic balance, the intracellular water comes out leading to reduction in the cellular volume. These changes cause mechanical damage to the cells. It is noted that the temperature range between 0 to -40 ^{0} C is most critical during freezing since most of the cryo injuries takes place in this temperature range.

During the process of cryopreservation, under gradual decreasing temperature, water freezes out of the solution and forms ice crystals until the attainment of the eutectic point. The super cooling, which is defined as the ability of the liquid to cool several degrees below its own freezing point, yet still remain in liquid state. This is an unstable state that often results in spontaneous freezing and formation of large metamorphic structures of ice crystals that may then puncture the cell membrane and destroy the cells The fluxing activities may be controlled to an extent during cooling procedure. The process of spontaneous freezing is referred to as nucleation. To prevent the formation of ice crystals, cells should be dehydrated as much as possible; however dehydration process only starts following nucleation. Seeding (inducing ice formation in a controlled area of the sample) the freezing solution when it is just below its normal freezing point can induce ice formation.

Dehydration may be achieved by decreasing the eutectic point of the cell in solution by adding a cryoprotectant with a lower eutectic point. Rapid freezing may lead to the formation of lethal size ice crystals, whereas slow freezing acts in reverse way. Large degree super cooling may be avoided by artificial seeding the sample at a temperature slightly below its freezing point.

CRYOPROTECTANTS AND EXTENDERS AND THEIR ROLE IN CRYOPRESERVATION:

Cryo injury can be reduced in presence of some protective chemicals called cryo protectants and a diluting solution, called extender which acts as the vitrification solution. During freezing, intracellular water should be allowed to come out of the cell. Rapid cooling fails to provide sufficient time for the cells to dehydrate and avoid the detrimental effects associated with intracellular ice formation. In contrast slow cooling results in excessive dehydration and reduction of cellular volume to the extent that is not reversible. Thawing should be done at a fast rate that it should not allow the ice crystal formation, when temperature is raised above – 40`c.

For long term storage, liquid nitrogen (-196 ^{0}C) is the most useful medium. However freezing at any temperature below -70 ^{0} C will generally maintain the viability of the cells for a longer period.

The osmotic property of cells regulates most of the chemical events related with freezing. Intracellular and extra cellular environments associated with water transport and membrane permeability form the basis behind cryopreservation of cells. Generally the larger cells that are less permeable should be frozen slowly than those that are smaller and more permeable should be frozen rapidly . The osmolality of a solution is a measurement of solute concentration and is a critical factor in the freezing process. This measurement defines the ambient environment of the cells under preservation and directs water movement across the cell membrane, which is crucial for the penetration of cryo protectant into the cells during freezing procedures. Cryo protectants help to prevent or reduce the intra cellular ice crystal formation during freezing and thawing.

A good cryo protectant should be non toxic, water soluble and capable of altering the physico chemical property during freezing.

There are two types of cryo protectants, permeating and non permeating. Glycerol, Dimethyl sulfoxide (DMSO), ethylene glycol, and methanol can permeate into the cells. The non permeating cryo protectants like polyvinyl pyrollidone (PVP), glucose, sucrose, egg yolk, serum and skimmed milk form a coating externally around the cell that prevents ice formation in its vicinity. The non permeating cryo protectant helps to depress the freezing point and prevents ice crystal formation in the vicinity of the cell. The adjuvant like egg yolk provides additional strength to membrane stability.The choice of cryo protectant and its concentration are of much importance.

EQUILIBRATION

The chemical components of the extenders like fish ringer solution, modified fish ringer solution are weighed accurately and dissolved in double distilled water. The cryo protective agents (CPA) like DMSO, glycerol are often used. The CPA should not be directly mixed with the milt sample. It is mixed first in the desired ratio and kept in under refrigeration . The resulting solution is called the cryodiluent. It is very important to maintain isothermal conditions while mixing the milt with diluent. The time interval between mixing the milt with the diluent and starting the freezing process is called equilibration time.; During equilibration, the CPAs generally enter into the cells and protect them from cryo injury during freezing. The time varies from species to species as the CPA toxicity time also depends on the equilibration time and temperature. It is often observed that longer equilibration times are toxic and deleterious for the survival of fish gametes.

PROTOCOL FOR THE CRYOPRESERVATION OF SPERMATOZOA IN SEA BASS

Preparation of Cryodiluents

5% Sucrose with 20% Glycerol or 10% DMSO or Modified Fish Ringer with 20% glycerol were found to be effective for Sea bass .

Extender 1: Modified Fish Ringer

NaCl - 0.65g

KCl - 0.30g

$NaHCo_3$ -0.02g

$CaCl_2$ - 0.03g

Double distilled water – 100ml

pH: 8.0

Extender 2: 5% sucrose in 100ml double distilled water

The chemical components of the extenders mentioned above are weighed out accurately, dissolved in the double distilled water to which the cryoprotectant is added

. The cryoprotectants used were DMSO and glycerol in the percentages specified above. The resulting solution is called the cryodiluent . The cryodiluents should be kept under refrigeration .

Collection of milt :

Milting males have to be selected . Milt from the live oozing males is collected with a micropipette , by pressing the abdomen. Care should be taken to avoid faecal contamination. Collected milt is stored in cryovials / tubes and kept in ice.

Adding the cryodiluent :

The milt is mixed with the cryodiluent in the ratio of 1:4 , i.e. to one part of milt 3 parts of the cryodiluent is added . A known volume of milt is

pipetted out into a clean dry cryovial and to that the cryodiluent is added and mixed well. Filling of the diluted milt in cryo vials is called ampouling.

5% Sucrose with 20% Glycerol or 10% DMSO or Modified Fish Ringer with 20% glycerol were found to be effective for Sea bass .

Equilibration

The time interval between mixing of milt and dipping them in liquid nitrogen is called equilibration time. The equilibration time varies from ten minutes to one hour according to the species and sample . In the present protocol an equilibration time of 30 minutes was followed.

Freezing

A two stage freezing protocol was followed.

1. Pre-chilling: The samples which were initially kept in ice are first exposed to liquid nitrogen vapors at a height of 4-8 cm from the level of liquid nitrogen in a ice box. The holding temperature will be about -60 to -70 ^{0} C. Pre- chilling is done for a period of 10 minutes and is done to prevent cold shock to the cells.

2. Dipping the samples in Liquid nitrogen. After pre-chilling the samples are dipped into liquid nitrogen by placing them in labeled canisters. One fourth of the canister is first filled with cotton, on which the cryovial is

placed. The canisters are then again plugged with cotton and immersed in the liquid nitrogen can. Liquid nitrogen levels are maintained by constantly replenishing, to compensate for loss through evaporation. Liquid nitrogen cans can be kept in a cold room to minimize loss through evaporation.

Thawing

The cryovials are taken out of the liquid nitrogen and thawed by dipping in luke warm water (35-40 ° C) for a few seconds. The activating solution used is sea water. Post thaw motility is judged by the percentage of motile spermatozoa, vigor of motility and duration of post thaw motility.

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PROCUREMENT, TRANSPORTATION AND ACCLIMATIZATION OF FISHES FOR BROODSTOCK DEVELOPMENT

A.R.Thirunavukkarasu and M.Kailasam

1. Introduction

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

2. Procurement

Adult Seabass can be procured from wild catch or from the farm harvest. Seabass is available in the rocky bottom areas, river mouth and backwater regions. The selection of a suitable gear or method of capture is very important in order to avoid injury to the fish. Seabass is caught using gill net, dip net, seine net and hook and line. Dip net is the best gear for brood stock fish collection because, dip nets cause less injury to the fish. But operation of this gear is not possible in many places. The most commonly used gear is the hook and line using live/dead shrimp or fishes as bait taking advantages of the preying tendency of the fish.

2.1. Criteria for selection of fishes for broodstock development

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

3. Transportation

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

Manual transportation from nearby areas is done by simply transporting the fishes using buckets or troughs with water covering with perforated lids in the case of small fishes. If the fishes are large, (more than 4.0 kgs in size), they can be transported using specially designed transport tubes. Large rubber tubes specialy designed with perforation for water exchange can be used. Fishes can be allowed into the tube and can be manually toed along the water-line to reach the hatchery. This would avoid injury to the fish.

4. Acclimatization

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

4.1. Quarantine

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

4.2. Conditioning of broodstock

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

5. Broodstock holding facilities

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

BROODSTOCK MAINTENANCE

M.Kailasam, A.R.Thirunavukkarasu and J.K. Sundaray

Introduction

Aquaculture of finfishes can be sustainable if the critical in put like seed, feed etc. are available with required quality in adequate quantity. The major problem in the coastal aquaculture of finfishes is the non availability of the seed. Many of the candidate species of finfishes breed in the sea and the larvae/fry are drifted to the backwaters, estuaries, and inland water bodies by the tidal currents and spend the growing phase in the confined conditions. However, the seed availability in the natural source is highly irregular, scarce and not dependable. Even if natural source is available, collection is a problem and the natural source will deplete which is not advisable. Hence for a viable farming, seed have to be produced in the hatcheries and supplied. To develop a technology for breeding and seed production, gravid fishes of both sexes are prerequisite. Locating the fully matured fishes from the spawning grounds, collection and transporting to hatchery for seed production is laborious. The gonadal conditions of the wild caught fishes also may be or may not be suitable for inducing them to spawn immediately. For hatchery seed production of finfish seed, a hatchery operator has to develop a viable captive broodstock from which manipulating the environmental conditions or using hormones, gravid fishes can be obtained for spawning and seed production.

Broodstock Development

Fishes of 2 – 7 kg size in the case of seabass, are collected either from wild catches or from the culture system and carefully transported, conditioned and after prophylactic treatment, released into the broodstock holding facilities and maintained.

Broodstock Holding Facilities

Seabass attain maturity when the fishes are in 3 – 4 years old. Females attain maturity when they are more than 3 kgs and males 2 – 2.5 kgs in size. This size group fishes are maintained in the broodstock holding facilities.

Broodstock can be held in cages, in ponds or in constructed concrete holding tanks.

Cages

The fish holding cages can be in the size of 50 – 200 m^2 with depth of 2 meters (i.e. in dimension of 5 x 5 x 2 m or 10 x 10 x 2 m). Floating net cages are preferred. Cages are made of polyethylene net webbing in the mesh size of 4 – 8 cms. Fishes are stocked @ kg/m^3. Care should be taken in the maintenance of the cages. Cages should be checked for damages and cleaned for proper water inflow daily. Fishes maintained in the cages with proper feeding and health management. Fishes can be transferred to other cages 2 – 3 months prior to spawning for inducing maturation and spawning. Cages can be set up in safer places where it cannot be damaged due to waves and tidal actions.

Ponds

Broodstock can also be maintained in well constructed ponds of 500 – 1000 m^2 area with proper water inlet and drainage facility. Pond depth should be 2 m and above. Pond water should be exchanged properly other wise, the infection can cause stress and further complication to the fishes.

Concrete tanks

Depending upon the production target, the number and size of the fishes proposed to be maintained broodstock holding tanks are constructed. It is advisable to maintain fishes in large tanks because the fish will have more natural condition and sufficient space will be there for swimming.

Broodstock tanks can be 50 to 100 tone net water capacity. In CIBA broodstock tanks of 12 x 6 x 2 m (with gross capacity of 144 tones and net water holding 100 tonns) are used. Broodstock tanks should have adequate water inlet and drainage provisions. Flow through facility is desirable and provision for aeration is advisable.

The pumping efficacy should be in such a manner that the broodstock holding tank can be filled within 1 - 2 hours and the drainage should be possible with ½ 1 hour.

The concrete tanks can be covered with shade nets to prevent the direct sunlight exposure to the tanks which facilitates the algal bloom.

Water Source

Water can be drawn either from the open sea or from borewells put up in inter tidal area where from water of the same salinity of seawater could be drawn. Open seawater source is desirable since it will be more natural than that of borewell water. However in certain months, when the nearshore water gets diluted due to freshwater drainage, the borewell water has higher salinity and during these months, borewell water can be useful.

Seawater well can be digged provided continuous water supply should be available according to the demand.

Water Quality Management

Broodstock fishes maintained in captive condition should be provided with environmental quality prevailing in the sea for maturation and spawning. Though it is not possible to provide all the conditions in the sea environment, atleast, the water quality to that of the natural seawater should be maintained to the maximum extent.

The desirable range of some of the water quality parameters in a broodstock tank are

Temperature	-	28 – 32°C
Salinity	-	28 – 33 ppt

pH	-	7.0 to 8.2
Dissolved oxygen	-	more than 5 ppm
Ammonia	-	less than 0.1 ppm
Nitrite-N	-	less than 0.01 ppm
Phosphate	-	10 – 20 mg/l
Suspended solids	-	2 – 5 mg/l

Water should be clear. Water drawn from sources can be filtered through biological filter or pressure sand filters for better water quality.

In tanks with over cover and with flow through system the algal growth in the broodstock tanks will be minimal. However tanks open to sky under direct exposure to sunlight is prone for algal growth. This algal bloom would reduce the visibility interfere in the feeding and also would reduce the dissolved oxygen content in the early hours of the day. In order to get rid of these problem as well as to remove the unfed feed and the metabolites, the tanks have to be cleaned thoroughly during early morning. After reducing the water level to the minimum level, bottom and the sides of the tanks are cleaned. Then water is refilled. By this process 70 – 80% of the water is changed daily in the broodstock tanks.

Stocking density

Fishes are maintained @ kg/m ^{3} in the broodstock tank. In cages the density can be doubled depending upon the water quality and feed management.

Feed Management

Maintaining of the fish with quality feed with nutritional value required for maturation and spawning will help in attaining the purpose for which the stock is maintained. As such, seabass is maintained feeding with trash fishes. Seabass is a voracious carnivorous fish feeding mainly on crustaceans/small fishes in live condition. But while maintaining in captive condition, the fishes have to be slowly weaned to feed on the diet that can be provided. In the broodstock holding tanks fishes are fed with trash fish @ % of the body weight in frozen form. Since the fish is adapted to feed on live fish it has to be gradually weaned to frozen fish. To start with, live and frozen trash fish are given in the ratio of 2 : 1 and slowly within a period 15 – 20 days, the live fish is replaced completely with frozen fish. Frozen fish feeding is desirable because it will reduce the labour of collecting live fish daily. The availability of live fish daily is also not dependable and for the live fish separate feed source have to be maintained. Moreover, the pathogens if any in the live fish will be transmitted to the broodstock. Fresh trash fish like Tilapia (*Oreochromis mossambicus*), Sardines (*Sardinella sp*), Horse mackeral (*Decapterus sp*) etc. are procured, cleaned and packed in polythene bags of 2 – 4 kgs block and stored in deep freezers at – 20°C. At the time of feeding, the fishes are taken out thawed, washed and fed to the fishes. It is important to provide trash fishes with addition of vitamin mix @ g/kg feed. Since squid is having rich resources of protein, Squid can be supplied to fish once in a week. Enrichment of broodstock feed helps production of good quality eggs which reflects in fertilization rate hatching rate and larval quality. Since seabass does not prefer to feed on the fishes settled at the bottom, care should be taken the fishes are on the sub-surface water column while feeding. The fishes are individually given if the size of the fish is small that could be

ingested by the fish, otherwise, the fish is made into pieces and given. Feeding is done once a day in evening hours. Excessive feeding should be avoided since the left over fish would deteriorate the water quality. If any fish is unfed it should be removed immediately.

Health Management

Since brood fish are maintained in higher densities compared to their distribution in wild the fish will experience stress. Moreover the fish are frequently disturbed while cleaning and observation for gonadal conditions. This will cause injuries leading to bacterial, fungal and viral infection. The natural pathogens, endemic to fish and pathogens in the water would also proliferate. These problems can be overcome by regular checking and proper prophylactic treatment in time. (Refer – health management for details.) It is better to have spare broodstock tanks, it can be used during dis infection of broodstock tanks. The broodstock tanks have to disinfected once in a month with chlorine to avoid cross contamination with parasite and bacteria.

Broodstock fish maintained are monitored for gonadal condition every fortnight. Maturing fishes are implanted with hormone pellet for accelerating the maturation and matured fishes are selected for induction of spawning. A well maintained broodstock fishes under controlled condition providing good water quality, feed and health will be the source for gravid fishes in 6 – 8 months of maintenance.

FISH BROODSTOCK HEALTH MANAGEMENT

S.V. Alavandi, N. Kalaimani, T.C. Santiago and A.R.T. Arasu

Indian brackishwater aquaculture has been dominated by shrimp farming, and the impetus for the finfish farming has been slow. While the past few years have witnessed heavy crop failures in shrimp farming due to serious disease problems, caused by viral epizootics, the Indian aquaculturists are looking for alternative species. The seabass, Lates calcarifer, was one of the candidate species of choice with its suitability for brackishwater farming, and the marked demand. Development of hatchery technology for the sea bass seed production by CIBA during late nineties has generated interest for diversification of aquaculture with this species.

As in other animal rearing systems, infectious diseases are among the greatest limiting factors, which affect sea bass culture, and the broostocks are no exception. Reports of various diseases and pathogens of sea bass have been documented from Thailand, Singapore, China, Malaysia, Australia, Philippines and India, and incidence of mortalities have also been reported. The causative agents of the diseases are parasites, bacteria, fungi, viruses, and less often diseases caused by non-infectious agents (Table 1).

Health Management

Control and prevention of infectious disease in aquaculture is a function of management. Incidence and severity of infectious diseases are very often dependent on the quality of aquatic environment in which the fishes live, quality of feed it consumes and the ease with which the fish are maintained. Health management in broodstock involves a holistic approach incorporating the following components:

1. Selection of healthy broodstock

Only healthy fishes should be selected as broodstock for further maintenance in the hatchery facility. Fish with obvious abnormalities such as hemorrhagic spots, wounds, damaged gills, skins, fins, scale loss etc should be discarded. Broodstock have to be screened for parasitic infestation and viral pathogens using reliable diagnostic tools during quarantine prior to their induction for breeding.

2. Quarantine Measures

It is essential to eliminate possible introduction of external pathogens as well as to heal wounds and abrasions caused by fishing and handling, through quarantine. The usual protocol includes a formalin (37-40%formaldehyde, HCHO) and malachite green (zinc free oxalate or aniline green) treatment and the treatment may be repeated if required after assessing the health status of the fish. Fish have to be held under quarantine for at least a couple of weeks. After the transfer of brood fish, the quarantine facility should be drained and thoroughly disinfected with a 500-ppm hypochlorite (NaOCl) solution.

3. Prophylactic treatments and maintenance of routine hygiene

Once the fishes are transferred to broodstock tanks, routine prophylactic treatments may be given to keep the animals healthy. Every batch of breeders should preferably be subjected to a periodical bath treatment, once in fortnight or once in a month. The commonly practiced prophylactic treatment for the broodstock include use of benzalkonium chloride (BKC), dichlorovos etc (Table 2).

Routine cleaning of hatchery and maintenance of hygiene in the facilities is of utmost importance in the fish health management. Tanks should be cleaned routinely to prevent the accumulation of organic material, and also to remove the free-living organisms.

- Cleaning of hands and footwear before starting work and, as and when necessary (disinfecting solution has to be replaced periodically)
- Cotton gloves may be used while handling fish.
- Tank bottom and inner walls must be periodically cleaned.
- Feed intake of breeders must be monitored carefully.
- Disturbing fish must be avoided as much as possible
- Each tank must be provided with separate tools in the hatchery and they must be disinfected (rinse and disinfect overnight with hypochlorite solution; rinse with freshwater or disinfected seawater and dry). Make sure that no disinfectant remains in the tanks, hoses and stones after cleaning.

4. Regular monitoring of fish health

Broodstock maintained in the regular rearing facility should be regularly examined for health status with gross observation and simple microscopic examination of gill/body smears. Periodic microbiological analysis may be carried out to assess the microbial load in the rearing water and inlet water.

5. Water quality management

All the important water quality parameters such as temperature, salinity, pH, dissolved oxygen, ammonia, nitrite etc should be monitored and recorded on a daily basis before water exchange. Recirculatory aquaculture systems would be useful in maintaining optimal water quality in the hatcheries.

6. Feed Management

Since the primary feed used for the sea bass broodstock is a forage fish, necessary precautions must be taken to prevent any introduction of pathogens or toxins. Only

quality live or frozen fishes free from parasites and other pathogens should be used as feed. Uneaten feed should be removed from time to time to prevent the putrefaction.

7. Chemotherapy

Therapy denotes treatment intended to restore the normal health of fish that is infected or diseased. Chemotherapy should be used only as a last resort in aquaculture. One must be very cautious while using antibiotics, since these can cause development of antibiotic resistant microbes in the aquatic environment and create tissue residue problems. Antimicrobial therapy is directed towards treatment of bacterial, fungal and parasitic infections and will not be useful in the treatment of viral diseases. Hence diagnosis of the problem plays a primary role in deciding such measures. Health status of the fish must be assessed by examining the gills, skin, feeding profile etc. prior to such treatments. If the therapeutant is new, a preliminary test before doing large-scale treatment must be always performed. Mode of treatment of affected animals can be through following ways.

i. Treatment via water:

Gloves and face covers must be used by the personnel to avoid contact with skin and eyes while applying the chemicals. Feeding should be stopped before 24 h of the treatment. Right dosage of the therapeutant must be calculated and the fish must be always treated with reduced water level. Treatment should be planned during the less stressful period of the day, usually in the morning. Plastic or glass utensils only may be used for mixing chemicals, since the metallic containers can react with the chemicals and form toxic substances. Treated fish have to be kept under observation to assess the health status. Incase of any emergency such as stress and abnormal behaviour, the treatment must be stopped and the treated water must be flushed out. Vigorous aeration must be provided during the treatment. Chemotherapy through water can be administered in any of the following ways.

- Dip treatment: In this mode of treatment, fishes are immersed in treatment medium, often with higher level of therapeutants, for a short duration (1-2 min)

- Flushing: This involves adding a specific amount of chemicals to the tanks and allowing it to flush through without interruption of water flow.

- Bath: Bath treatment of fish is done in confined and easily controlled volume of water. The treatment exposes the fish to desired concentrations of chemical for a short-term or a specified period of time without any flow of water. Once the treatment is over the treated water is flushed out quickly and replaced with fresh water.

ii. Treatment via feed

Treatment via water may not be always effective, especially against the endoparasites and bacterial infections. In such cases, appropriate therpaeutant can be administered into the fish through the feed. Antibiotics and other antimicrobial preparations can be administered through feed. Prior to application of antibiotics, it is always advisable to understand the antibiotic sensitivity pattern of the bacterial pathogen. Proper assessment of the disease situations and identifying the right therpaeutant and right dosages, at the right time are required to get successful results.

iii. Injection

This mode of treatment is feasible and useful for valuable stock of fish such as broodstock. Fish have to be anaesthetized or sedated prior to injection treatment.

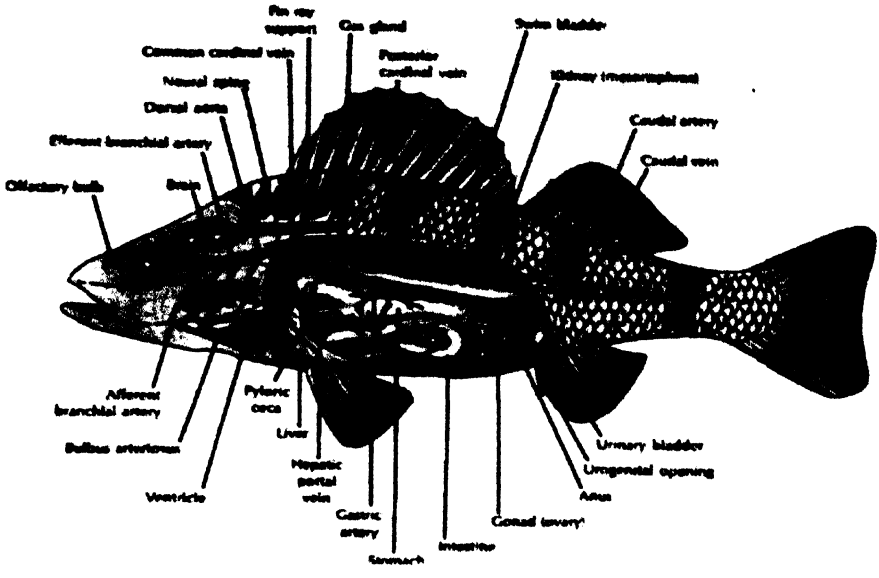
8. Disease diagnostics

Disease diagnosis using proper diagnostic methods is one of the important components in the health management. In most cases fish farmer may not have the level of expertise or facility for proper disease diagnosis, hence it is always advisable to take an expert opinion from a fish health specialist in the event of disease occurrence. However, basic knowledge about sample collection and preservation can be very much useful for submitting the samples to fish health laboratories and disease management decisions.

Fish tissues routinely used for detailed examination are gills, liver, heart, spleen, kidney and gastrointestinal tract (figure 1). Other tissues such as eye, swim bladder and brain may also be used for investigation as required. Squash preparations using these tissues are useful for locating microsporidians, myxosporidians and mycobacteria.

Small fishes can be fixed as whole, while the individual samples of organs/tissues can be fixed either in neutral buffered formalin or Bouin's fluid. Generally the tissue to fixative is 1:10 in volume. i.e., 1 g of tissue needs 10 ml of fixative for 24 - 48 hours at room temperature. The tissues should then be transferred to 70%ethyl alcohol until processing by routine histopathological techniques.

Figure 1. Anatomy of fish



Fresh tissues for nucleic acid based diagnosis can be collected in 70-95% ethanol/methanol or propanol.

Blood and kidney samples may be collected for isolation of bacterial pathogen if bacterial infection is suspected. Blood sample can be drawn from caudal vein using a sterile needle and syringe. Kidney sample must be taken aseptically after careful dissection. Samples for bacteriology can be inoculated on suitable culture media such as tryptose soya agar (TSA) and thiosulfate citrate bile salts sucrose (TCBS) agar for the recovery of bacterial pathogens. The bacteria isolated from these samples can be identified based on their morphological and biochemical characteristics with the help of Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984).

Table 1. Suggested treatment for infectious diseases of fish

PATHOGEN	CLINICAL PICTURE	SUGGESTED TREATMENT
Viral infections		
Viral Nervous Necrosis (VNN) caused by Nodavirus (icosahedral, 20-30 nm)	Loss of balance, abnormal swimming, lethargy and anorexia, extensive vacuolation of brain and retina, 90%mortality	Prevention by disinfecting the fish eggs using iodine and rearing with filtered sea water
Lymphocystis disease caused by Iridovirus (icosahedral, 130-330 nm)	Clusters of shining nodules on skins, gills and fins, lethargy, not lethal in adults	None reported
Bacterial Infections		
Vibrio sp., Vibrio parahaemolyticus, V. alginolyticus, V. anguillarum, Pseudomonas spp. Pasteurella spp	Loss of appetite, lethargy, hemorrahagic spots on body surface, fin and tail rot, Heavy mortality	Oxytetracyline, Nitrofurazone, Quinolones
Myxobacterial infection (columnaris disease) Flexibacter spp. Cytophaga marinus, C. columnaris	Fin (caudal, dorsal) and gill rot, sloughing of muscles, skins and fins, ulcerative and necrotic lesions, may cause 100%mortality	Tetracyclines
Streptococcosis caused by Streptococcus sp.	Lethargy, abnormal swimming, hemorrhagic cornea, exophthalmus	Tetracyclines
Parasitic diseases		
Protozoans		
Trichodinasis: Trichodina spp	Ectoparasite on gills and skin	Formalin and Acriflavin bath
White spot disease: Cryptocaryon spp	Ectoparasite on gills and skin, whitish or grayish spots on the skins or gills	Formalin
Velvet disease: Amyloodinium ocellatum	White spots on skin and gills, Tissue necrosis, abnormal swimming	$CuSO_4$
Myxosporeans	Anaemia	
Worm infestations		
Monogeneans: Diplectanum spp (Diplectanidae), Gyrodactylus spp (Gyrodactylidae)	On gills, on skin, heavy mortality	Formalin
Digeneans: Bucephalus sp., Lecithochirium sp., Pseudometadena celebesensis, Prosorhynchus sp.,	Skin, fins, muscles, retarded growth	
Crustacean infections		
Caligus spp	Gills, skins and oral cavity, Hemorrhagic/ulcerated	Neguvon
Ergasilus sp.,	On gills and body surface, Necrotic gills	Dipterex
Lernanthropus spp	On gills	

Table 2. Therapeutic chemicals used in fish health management

CHEMICAL	PATHOGEN	COMMENTS
Benzalkonium chloride (BKC)	Bacterial gill infection	A quaternary ammonium compound. A powerful disinfectant with added detergent action. Low dose bath treatment
Chloramine T	Ectoparsites, and myxobacteria	Low dose, prolonged immersion
Formailin, as 34-40%w/w Formaldehyde solution	Ectoparasites, Protozoans, Flukes	Formalin solution with a white precipitate should not be used. Formalin has deoxygenating effect, hence use aeration during treatment
Dichlorovos	Ectoparasites	Bath treatment.
Malachite green (zinc free)	Fungal infection and some Protozoa	Bath treatment Can be used as prophylactic treatment after handling
Potassium permanganate	Protozoa	Dip treatment as a prophylactic treatment
Levamisole	Nematode	Bath
Mebendazole	Tape worms	Through feed
Antibiotics	Bacterial infection	After testing the antibiotic sensitivity, only as last resort

Table 3. Broodstock prophylactic treatment

Therapeutant	Dosage	Frequency
Formalin	200 ppm for 1 h	Fortnighly
plus Malachite green	0.2 ppm for 1 h	Fortnighly
Dichlorovos	1 ppm for 30 min	Monthly

Further reading

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MOLECULAR DIAGNOSIS OF FISH NODAVIRUS WITH SPECIAL REFERENCE TO PCR

M.Poornima and T.C. Santiago

Introduction

Increasing disease problems mars the growth of fish production both nationally and internationally. Vulnerability of the fish production system to disease is due to the co-existence and close interaction of host, pathogen and environment. A small shift of equilibrium between these three can trigger a disease outbreak leading to mortality resulting in crop failure. Disease problems are inevitable, as aquaculture has to look forward to produce more animal protein, more jobs and more revenues for the people. To tackle the menace of disease problems, a scientific health management approach has to be developed emphasizing the conventional wisdom - 'prevention is better than cure'. An integral part of such a program is the use of diagnostic tests at the strategic point of production cycle to eliminate or control the disease causing pathogens.

Conventional diagnostic methods

The conventional diagnostic methods practiced in aquaculture are mostly adapted from the field of human health and veterinary sciences. Visual examination, microscopic, histological examination and bacterial examination are the most widely used and still form the essential part of disease diagnosis(Table 1) .But these methods often fail to deliver data in time to support a decision making in the health management to salvage the crop. This is mainly due to the time consuming and laborious methodologies and the inability of these tests to detect sub-clinical / latent / carrier state of infection.

Table 1: Methods available for disease diagnosis and pathogen detection

Method	Tests and data obtained
History	History of disease at facility or region, facility design, source of seed, type of feed used, environmental conditions etc.Gross, clinical signs, lesions visible, behaviour, abnormal growth, feeding or food conversion efficiency, etc.
Direct microscopy	Bright-field, phase contrast, or dark field examination of stained or unstained tissue smears, whole-mounts, etc. of diseased or abnormal specimens
Histopathology	Routine histological or histochemical analysis of tissue sections
Electron microscopy	Ultrastructural examination of tissue sections, negatively stained virus preparations, or sample surfaces
Serological methods	Use of specific antibodies as diagnostic reagents in immunoblot, agglutination, ELISA, IFAT, or other tests.
Tissue culture	In vitro culture of pathogens in cell lines
PCR	Amplification of unique portion of a pathogen's genome to readily detectable concentrations using specific primer pairs

DNA - based diagnostics

Developments in molecular biology enabled researchers to collect information on the genetic material that serves as the blueprint for all living organisms. The most recent development in diagnostics have utilized molecular biology to design new generation of diagnostics tools, the Polymerase Chain Reaction (PCR) and Gene Probes. These DNA based diagnostic tools stand out among other conventional diagnostic methods with its speed, sensitivity and simplicity. PCR is relatively a simple technique by which a DNA or cDNA template is amplified many thousand or a million fold quickly and reliably in a short period of 3-4 hours.

PCR - based diagnosis of nodavirus

Viral encephalopathy and retinopathy (VER) or viral nervous necrosis (VNN) caused by nodavirus infection is recent viral disease problem especially brackishwater fish farming. Even though the virus affects 30 different fish species, the greatest impact is seen in sea bass (Lates calcarifer). It has been reported as a serious disease of larvae and juvenile fish. Till date, no treatment is known to control the nodavirus Hence, early diagnosis followed by suitable management practices is the only alternative in tackling this disease. Diagnosis of nodavirus can be done by methods such as histopathological and ELISA techniques. These methods can detect the nodavirus infection only in the late stage of infection. The PCR is a powerful and sensitive diagnostic tools for identification of viral pathogens even at a very early stage (asymptomatic / carrier stage) of infection.

PCR Primers for Nodavirus

The 2 primers, a reverse primer (5'-CGA-GTCAAC-ACG-GGT-GAA-GA-3') and a forward primer(5'-CGT-GTC-AGT-CAT-GTG-TCG-CT-3'), used are for amplification of a target sequence (430 bases) of theRNA2 (Nishizawa et al. 1994).

PCR – Protocol

PCR and agarose gel electrophoresis are used in conjunction to determine the presence or absence of nodavirus in fish. The standard operation procedure consists of:

I. RNA – template preparation / nucleic acid extraction

Pool the larvae or head portions of the moribund juveniles and fix in absolute ethanol. Total RNA extraction from tissue with TRIzol reagent is as per the manufacturer's protocol, will be. In brief, homogenize the tissues with 900 µl of TRIzol reagent and incubate for 5 minutes at room temperature. Add two hundred microliters

of chloroform to the suspension, incubate for 2 to 3 minutes at room temperature, and then centrifuge at 12,000 x g for 15 minutes at 4°C. Add 500 µl of isopropanol to the RNA in the aqueous upper phase. Incubate the mixture for 10 minutes at room temperature and then centrifuge at 12,000 x g for another 10 minutes at 4°C. Wash the pellet obtained after centrifugation 1 ml of 75% ethanol and centrifuge at 7,500 x g for 5 minutes at 4°C. Air dry the RNA pellet and dissolve in 50 µl of diethylpyrocarbonate-treated water.

II. PCR preparation and RT-PCR reaction

1. Prepare a master mix of the following components aliquot into individual 25 µl PCR reaction vials. To each PCR reaction mix containing dNTPs, $MgCl_2$, RNase inhibitor, add template RNA (5ng/µl) for reverse transcription using murine leukemia virus(MuLV) reverse transcriptase. Incubate at 42^0C for 30 min. Place the reaction tubes in thermal cycler, run PCR using the forward and reverse primers with the following programme:

2. Program thermal cycler as follows:

1 Cycle of	:	94 °C for 3 minutes
35 Cycles of	:	94 °C for 1 min
		58 °C for 1minute
		72 °C for 1minute
1 cycle of:		72 °C 10 minutes

The amplified product was visualised using 2% agarose gel electrophoresis.

Agarose Gel Electrophoresis

1. Assemble large gel electrophoresis box to run electrophoresis.

2. Prepare a 2.0 % agarose gel as follows: weigh the agarose in an Erlenmeyer flask. Add 1X TBE to the weighed agarose. Boil the agarose to dissolve. Once the agarose is dissolved, remove the flask and left at room temperature to cool.

3. Once the agarose solution cooled to about 50°C, add 0.1µl ethidium bromide, mix well, then pour the solution gently to cover the entire

surface of the platform and leave undisturbed for about 15 minutes.Once the gel is formed, remove the comb gently .

4. Cover gel with 1 X TBE and load samples. Load 5 µl of PCR marker into one lane of the gel. Run the gel at 80 volts for about 1 hour, or until the loading dye lane runs off the anode side of the gel.

5. Place gel on a UV transilluminator to visualize PCR product and photograph.

Conclusion

Molecular diagnostic technique like PCR provides a fast, sensitive and specific tool for disease diagnostics in aquaculture. The PCR has a wide and significant use in checking the entry of lethal pathogens like virus to the hatchery and growout system by helping to select healthy broodstock and seeds. This can also be used in the epizootiological study of the pathogen in an effort to draw disease control measures.

BROODSTOCK NUTRITION AND FEED MANAGEMENT

M. Natarajan

Introduction

Nutritional status of the broodstock greatly influences the reproductive performance of all fishes. The broodstocks of Asian Sea bass, *Lates calcarifer* should also be maintained in good nutritional condition so that their breeding performance such as gonadal growth, maturation, spawning, fecundity, hatching percentage and larval survival and growth are not compromised or decreased. Fertilised eggs are completely dependent on the yolk nutrients for larval development till they hatch and mouth-opens for start exogenous feeding. Therefore all the required nutrients for larval growth and metabolism should be packed into the ovulated egg. These materials are synthesized and placed in the egg during the process of vitellogenesis, which occur prior to ovulation in the females. On the other hand for production of sperms in males, the energy required is considerable less. Diana (1983) estimated that in pike (*Esox luscious*, a freshwater carnivore), the annual reproductive effort of males aged between 1 and 3 years was 7 to 10% and for females, it was 14 – 16%

Feeding Rate

Research on Rainbow trout have shown that ration size and feeding rate influence the size of eggs, number of eggs produced per female and quality of eggs produced. Good feed should be provided during the months prior to the spawning season for proper build-up of energy reserves for vitellogenesis in females and spermiation in males. In Asian Seabass which has an extended spawning period, feeding of spawners much be continued at least once a day during the spawning season also.

Essential Dietary Nutrient for Broodstock

The major dietary components of the broodstock diet that play important roles in reproductive performance of marine fishes are –

(a) Proteins
(b) Essential fatty acids
(c) Vitamins
(d) Carotenoids / pigments
(e) Phospholipids and
(f) Minerals

Generally, the optimal dietary protein level for growth is also the optimal level for reproduction. This has been demonstrated in *Oreochromis niloticus* (DeSilva & Radampola, 1990). Amino acid requirements of broodstock fish are also generally similar to that for optimal growth. In a classic study of Red sea bream, *Pagrus major*,

Watanabe *et al.* (1984) found that a dietary protein level of 45% was optimal for (a) number of egg produced, (b) number of viable eggs and (c) number of larvae hatched out. They also demonstrated that replacing white fish meal with cuttle fish meal, remarkably increased egg viability, hatching rate and percentage of normal larvae (Table 1). Since dietary protein quality has a significant influence on the success of reproduction, broodstock diets should contain good quality protein. Similarly, Cumaranatunga and Thabrew (1989) have shown in Nile tilapia that feeding fishmeal resulted in better ovarian growth and larger oocytes than legume meal. This is due to higher levels of vitellogenic proteins and lipids in fishmeal.

Marine oil supplements are necessary in broodstock feeding. Fish are incapable of synthesizing linoleic (18:3 ω6), linolenic (18:3 ω3), arachidonic (20:4 ω6), eicosapentaenoic (EPA) (20:5 ω3) and docosahexaenoic (DHA) (22:6 ω3) highly unsaturated fatty acids and are therefore Essential Fatty Acids (EFA). Moreover, unlike fresh water fishes, marine fish including Asian Seabass, are incapable of elongating the shorter chain fatty acids. Since these HUFA affect growth and survival of fishes, the quality of the eggs are also affected if the mother fish receive EFA deficient diets. EFA deficient diets fed to red sea bream gave very few buoyant eggs (Table 1). Similarly, substitution of corn oil in place of squid liver oil deteriorated egg quality. In case of *Siganus guttatus*, fishes that received Pollack liver oil in their diet laid eggs for more than five months but did so only for two months when fed diets without Pollack liver oil.

Fish eggs contain a large amount of phospholipids in their yolk. It is therefore advisable to include a source of phospholipids such as lecithin in the diet of broodstock.

Vitamin E plays an important role in reproductive physiology in fish as it does in birds and mammals. This has been confirmed in freshwater Ayu (*Plecoglossus altevilis*), Carp (*Cyprinus carpio*) and rainbow trout broodstocks. Dietary vitamin E in the broodstock diet is mobilized and transported to the oocytes wherein it greatly helps in hatching and survival of the young. The concentration of this vitamin is high in the eggs and low in the tissues of the female spawner after breeding. During larval development, the level drops rapidly. Hence the importance of dietary vitamin E is more during early maturation of the female fish and less critical during final maturation and spawning activity. The Gonado-Somatic Index in carp fed α-tocopherol deficient diet was drastically reduced and the ovaries contained oocytes without yolk granules or yolk-vesicles.

Low and deficient levels of trace elements such as manganese, zinc and iron lowered the percentage of both eyed and viable eggs in rainbow trout (Takeuchi *et al.*, 1981). Phosphorus deficiency in red sea bream broodstock resulted in increased deformities in the larvae (Watanabe *et al.*, 1984).

It has been demonstrated that if the parent fish are fed diets containing pigments such as β-carotene, canthaxanthin, asthaxanthin or natural pigment sources such as krill oil there is good improvement in the egg quality, particularly if fed just prior to breeding (Table 2). This is particularly applicable for fishes that continue to accept feed even while breeding. A summary of the known nutritional requirements of fish broodstock is given in Table 5.

Feeding of Seabass Broodstock.

In Malaysia, Seabass (*Lates calcarifer*) spawners are fed small low-grade fish (not trash fish) at a rate of 2 %body weight once daily. These small fishes are without spines and with soft scales. The feed fish is washed and rinsed with the head portion and intestine intact and fed to broodstock (Ali, H. M., 1986). Ponds stocked with seabass were given trash fish once a day (afternoon) by Kuo (1984). Kungvankij (1986) fed seabass brood fish with fresh cleaned trash fish given daily at the rate of 5 %f total biomass. Maneewong (1986) mentioned that sardine or anchovy with intestine and head removed are used for feeding after chopping into bite-size pieces at 1 %f body weight once a day in the morning.

At Tahiti, French Polynesia, induced spawning and larval rearing of *Lates calcarifer* is being done since 1988 following IFREMER technology. Here the spawners are fed a 'Semi-Wet' food composed of fresh products of high nutritional value supplemented with fresh tuna fish. The feed is provided three to four times per week till the fishes are satiated (2%body wt.). The feed is reported to contain 42%rotein, 8 %lipids, 36%noisture , 9.6%inerals (Gilles Le Moullac *et al.*, 2003) and the digestible energy content of this feed is 15 MJ/Kg(Thouard *et al.*, 1994).

In the case of European Seabass, *Dicentrarchus labrax*, feeding squid a few times each week starting two months prior to spawning help in obtaining high quality eggs. Dietary lipids have been found to be important because long-term deficiencies in ω3 HUFA can induce early gonadal atresia, lower fecundity and subsequent reduction in egg survival. Other trace nutrients such as vitamin C and E and carotenoids are also very important. The overall requirements for trace nutrients are satisfied by a mix of high-quality food items such as fresh squid, cuttle fish, shrimp, krill and fish. Marine lipid and vitamin supplements may be included. Although semi-moist and dry compounded diets are sometimes used as maturation diets for this species, they are generally accompanied by a fresh component such as squid, shrimp or fish. The composition of experimental broodstock diet for seabass and grouper (Meyers, 1987) is given in Table 3 and European Seabass in Table 4..

At CIBA's Muttukadu Field Centre, Seabass are maintained in 100 tonne RCC tanks and fed *ad lib* on frozen whole tilapia. Feeding time is usually after tank cleaning and water exchange (11.00 – 12.00 hrs). The excess food, which falls to the bottom of the tank, is removed.

Conclusion

Fresh diets offered to broodstock of fish and shrimp have many disadvantages but they are indispensable at the present level of knowledge. Most of the cultivable aquatic species of interest are cultured for market exclusively on artificial formulated feeds. However, when they are maintained for breeding purposes the emphasis is on the use of natural fresh feeds. The fastidious nature of the species concerned and the many unknown nutrients in natural feeds has made their use a regular feature in broodstock husbandry. The use of compounded dry feed can at the most be up to 50%nly, without affecting performance. The overall husbandry conditions also, such as, stress, water quality, rearing temperature, photoperiod and management procedures

in addition to the nutritional aspects, determine the success of the broodstock in terms of reliable seed productions.

Table 1: Effect on the spawning and egg quality of red sea bream *Pagrus major* of broodstock diets of different composition (Watanabe *et al.*, 1984).

	Control	Low protein	Low phosphorus	EFA deficient	Cuttle fish meal
	White fish meal 45 % CP				
Egg					
Egg produced (x 104/fish)	100.5	72.7	84.1	116.5	173.5
Buoyant egg %	80.9	54.4	62.1	23.9	88.5
Abnormal egg %	30.7	70.7	67.9	93.7	2.7
Av No. of oil globules	1.7	3.5	3.1	6.2	1.0
Hatched larvae					
Rate of hatching %	69.4	23.6	26.3	0.9	93.9
Deformity %	23.3	84.1	75.5	-	1.9
Normal larvae %	62.4	3.8	6.2	-	97.6
Final productivity of fish seed from total eggs produced %	24.3	0.1	0.3	-	78.9

Table 2: Effect of broodstock diets of different composition fed before breeding season on the spawning and egg quality of red sea bream (Watanabe *et al.*, 1984).

	High protein (fish meal)	Fish meal + Cuttle fish meal	β-Carotene + Canthaxanthin	Krill oil extract	Frozen raw Krill	Corn oil
	55 %P, 10 %lpid	45 %P, 10 %lpid	0.1 % 0.3 %	9 %		10%
Egg						
Egg produced (x 10^4/fish)	149.5	121.6	120.4	90.1	202.1	58.7
Buoyant egg %	49.1	68.6	56.4	69.6	82.7	18.2
Abnormal egg %	77.5	22.1	37.0	20.9	8.1	94.0
Av No. of oil globules	2.4	1.5	1.8	1.2	1.1	3.4
						Hatched larvae
Rate of hatching %	83.1	93.7	77.4	67.5	90.3	27.3
Deformity %	14.8	4.7	15.0	8.4	2.0	43.2
Normal larvae %	51.6	82.2	74.3	88.2	91.2	24.0
Final productivity of fish seed from total eggs produced %	21.1	52.8	39.1	41.4	68.1	1.2

Table 3: Ingredient composition of experimental broodstock diet for seabass and grouper (Source: Meyers, 1987).

Ingredients	Level of inclusion	
Fresh ground fish	46	%
Fish meal (local)	20	%
Extracted soybean meal	12	%
Wheat pollards	4	%
Fresh mussel meat	4	%
Fish oil	4	%
Soybean lecithin	3	%
Vitamin mixture	2	%
Seaweed binder	5	%
Composition of vitamin mixture		
Thiamine HCl	120	mg/kg dry diet
Riboflavin	40	mg/kg dry diet
Pyridoxine HCl	120	mg/kg dry diet
Cyanocobal amine	0.02	mg/kg dry diet
Folic acid	5	mg/kg dry diet
Niacin	150	mg/kg dry diet
Calcium pantothenate	100	mg/kg dry diet
Biotin	2	mg/kg dry diet
Vitamin C (Sodium ascorbate)	1000	mg/kg dry diet
Inositol	800	mg/kg dry diet
Choline chloride	1200	mg/kg dry diet
Vitamin A	5000	IU
Vitamin D	1000	IU
Vitamin E	200	mg/kg dry diet
Vitamin K	40	mg/kg dry diet

Table 4: Proximate composition of experimental European Sea bass broodstock diet (expanded 9 mm pellet)(Bruce *et al.*, 1999).

Moisture %	3.4
Protein %	51.4
Lipid %	20.3 *
CHO	27.8
Ash	10.2
Fibre	1.2

*Oil component (Tuna orbital oil) sprayed after the pellets are extruded.

Table: 5 Summary of the known nutritional requirements of fish broodstock. [Source: Kanazawa (1988)]

Species	Requirement
Ayu (*Plecoglossus altevilis*)	Vitamin E increases spawning success, egg survival, hatchability and larval survival. Requirement in broodstock is 34 mg/kg diet. Phosphorus increases spawning success. 20:5(ω3) and 18:3(ω3) fatty acids are probably required.
Carp (*Cyprinus carpio*)	Vitamin E increases GSI and is required for vitellogenesis and for proper maintenance of ω6 fatty acids in oocytes. ω3 fatty acids are probably required.
Rainbow trout (*Oncorhynchus mykiss*)	Based on transfer of nutrients to eggs, requirements are 10000 – 20000 IU/kg diet for vitamin A, 100 mg/kg diet for Vitamin E although lower for vitamin D. Low-protein/high-energy diets are as good as high-protein/low-energy diets for broodstock development. Trace minerals, particularly manganese, are required. 20:4(ω6) and 18:2(ω3) fatty acids are EFAs for broodstock. EFAs are also required for high quality sperm.
Red sea bream (*Pagrus australis*)	Vitamin E is required and probably has the same effect as for ayu. ω 3 fatty acids are required for buoyant (viable) eggs. β-Carotene and other carotenoids are important for egg viability. An unknown component in cuttlefish meal enhances spawning success.

INDUCED MATURATION

M.Kailasam, A.R.Thirunavukkarasu and J.K. Sundaray

The reproductive activities are integrated with seasonal – environmental cycles in most of the aquatic species. The environmental exogenous factors such as temperature, photoperiod and rainfall, along with the endogenous physiological cycles send signals to the neuroendocrine system; which in turn regulate the pituitary – gonadal functions. If fishes kept under captivity in confined tanks for breeding, the change of environment from natural sea to a hatchery tank / pond can have a significant effect on hormonal regulation of gonadal function. The most common problem in the captive land based broodstock is the inhibition of final stages of oocyte and sperm development though early maturation takes place. The release of these gametes is referred to as spawning. Individual variation in terms of maturation among the brood stock fishes of seabass under captivity is observed. Some of the fishes attain mature gravid condition without any hormonal intervention and some did not.

Seabass is a protoandrous hermaphrodite fish. They are males during early stage of its life cycle and become females in later period. Reproductive system is very much complicated in hermaphrodite fishes since they go through different phases of hormone secretion which is responsible for gonadal development.

Maturation process can be induced/ accelerated either by simulating the environmental conditions prevailing in sea or through the administration of the hormones responsible for maturation and spawning. However, simulating sea environment in many cases is difficult.

Hormonal Manipulation

The knowledge on the endocrine mechanism and the reproductive hormones is essential for inducing the maturation process under captivity. Maturation requires stimulation by chronic and slow increase in hormone levels. This sustained slow release hormone delivery can be enhanced through external sources in two ways:

I. Hormone Through Feed

Although most common and easy method of administration is through feed;it has certain limitations.

a. Only hormones that are not susceptible to enzymatic degradation in the digestive tract can be used.

b. There will be loss or hormone while this feed is in water.

c. No guarantee for absorption of hormone across the wall of intestine.

d. No control over the dosage administered as it depends upon the feeding rate of the individual fish.

e. Due to the above mentioned losses;excessive hormone has to be given.

f. Fish will revert back if hormone is not supplied continuously.

II. Hormone Through Implantation

Search for chronic hormone delivery mechanism has produced a variety of pellets and capsules which help in slow release of hormones, when implanted into the musculature or abdominal cavity. These implants would assist in overcoming the short bioactive like of the hormones and induce constant elevation of gonadotropin secretion accelerating the final maturation of gonads. This technique has both advantages and disadvantages.

1. High risk is involved for life of the fish since implantation has to be done by making incision.

2. Sustained release of hormone makes the fish to respond continuously.

3. Implantation has to be repeated whenever necessary.

Therefore it is important to study the reproductive stages of these fishes before application of hormones.

Adopting the following procedure can do the induction of maturation process in seabass:

1. Selection of Breeders

Breeders have to be selected from the captive broodstock before the onset of the breeding season, so that they can be conditioned to the environmental and diet controls. Breeders selected should be active, fins and scales should be complete, free from diseases, parasites and wounds. Males and females are selected based on their respective gonadal maturation stage. The maturity assessment of the fishes is done by ovarian biopsy as given below:

1. The fishes in the broodstock tank are secured individually in small cloth hapa for observation. In case the fishes are agitated too much or if wild broodstock fishes are procured, they can be transferred to anaesthesia tank containing sea water with 250 ppm of anaesthetic (MS – 222 or 2-phenoxy ethanol). Otherwise a cloth bag or perforated plastic hood can be covered over the head upto the pectoral fin so that the fish would become docile.

2. After turning over the fish; abdomen is gently massaged in head to tail direction. If milt extrudes out of the genital pore, it is a ripe male.

3. If milt is not there, a polyethylene canula of 1.2mm diameter is inserted into the genital opening of the fish upto 10 cm. The other end is aspirated gently by mouth while withdrawing the canula slowly.

4. The contents are collected in a watch glass and observed microscopically. Measuring the ova diameter using an ocular micrometer assesses the stage of maturing.

5. Female with 0.4 mm average egg diameter can be chosen for pellet implantation and the males without milt are also chosen for pellet implantation. Females tertiary yolk globule stage or a ova diameter of 0.45 – 0.50 mm are chosen for breeding purpose.

2. Pellet Preparation

The sexual maturation in female seabass can be accelerated by implanting Leuteinizing Hormone Releasing Hormone – analogue (LHRH – a) pellet. The

hormone is incorporated in a matrix of cholesterol powder and made into a pellet of required diameter for implantation. Before the pellet preparation, fish is weighed and the dose is calculated accordingly. Dose of LHRH-a to be implanted is calculated @ 0.1 mg/kg body weight of the fish. Suppose three fishes of 4kg, 5kg and 6kg are to be implanted the combined requirement @1mg/1kg body weight would be 1.5mg.

a. Weigh the cholesterol powder @2g per mg of hormone and take in a clean, dry petridish.

b. Dissolve each milligram of the hormone in 0.2ml of 80% ethanol. Draw this the solution with a syringe.

c. Mix the cholesterol powder with dissolved hormone with a spatula. Equal quantity of pinch of cellulose or gum acacia is added as a binder.

d. Weigh the total amount of powdered mixture containing the hormone required for each fish.

e. Compact the powdered mixture into cylindrical pellets by using pellet maker of required diameter, usually 0.2-0.3mm.

f. Dry the pellet in hot air over at 37°C for 2-3 hours or at room temperature for a day and stored in capped vials.

LHRH-a or MT (17-α methyl testosterone) pellets can also be prepared in the above mentioned manner and used for the induction of maturation in males. This case is applicable only if adequate males are not available. By treating the females through methyl testosterone it can be converted into males.

3. Pellet Implantation

I. Intramuscular implantation

a. The fish to be implanted is isolated and transferred to a anaesthesia tank, if agitated too much.

b. Turn the fish laterally and removed one or two scales with a forceps at a point 2-3 cm below the dorsal fin.

c. A short incision of around 1 cm width and 1 cm depth is made by using a surgical blade. Incision is made perpendicular to the normal spread of the muscle fiber.

d. The pellet is inserted into the musculature with the help of a forceps through the incision made and twisted at 90°C angle to get itself embedded in the musculature.

e. After withdrawing the forceps, the incision is sealed with pure Vaseline and the fish is given antibiotic treatment to prevent infection.

f. The fish is released in the broodstock tank after properly tagging for identification.

II. Intraperitoneal Implatation

a. Turn the fish on its back and remove few scales with a forceps at a point 7-8 cms ahead of the anus to expose the flesh.

b. A short incision of around 0.5 cm width; but deep enough to puncture the tissue lining of the body cavity is made. Care should be taken not to puncture any internal organ which would be fatal.

c. Fit a metal trochar into an 8 cm long plastic drinking straw and insert into the incision.

d. Withdraw the metal trochar implanter leaving about 3 cm of plastic straw guide protruding from the incised wound.

e. Drop the hormone pellet into the plastic straw guide and replace the trochar implanter to push the pellet into the body cavity of the fish.

f. Pull out the implanter and plastic straw guide from the incision.

g. Apply antibiotic Ointment and antiseptic Vaseline over the incision.

h. The fish is released in the broodstock tank after properly tagging for identification.

Hormone pellet implanted fishes are examined fortnightly to keep track of the gonadal development. Normally within 2-3 weeks after implantation, maturing fishes attain gravid condition. If needed; implantation can be repeated fortnightly/monthly once until the onset of breeding season.

LARVAL REARING

A.R.Thirunavukkarasu and M.Kailasam

1. Introduction

Rearing of hatchlings through various developmental stages providing required environmental parameters and feed is the most important phase in the seed production technology. This is still more significant in marine fishes like seabass. Seabass larval phase extends upto 21 days and during this time, the feed requirement, type of feed, quantity etc. also vary with every stage. The larvae have to be provided with nutritionally balanced diet. Moreover seabass larvae have the behavior of cannibalism. Differential growth is noticed even from the very early stages in seabass. The larger ones will eat the smaller ones in the rearing tanks, ultimately reducing the survival rate. Production of healthy fry depends upon taking care of all these aspects in the larval growing protocols.

2. Larval Rearing Tanks

Larval rearing can be done in indoor and outdoor tanks. Indoor tanks are desirable since close monitoring of feed, water quality and health is possible. The influence of extraneous factors like light intensity, algal blooms can be avoided. Outdoor tanks are mainly extensive type. Though the larvae may grow large, the ultimate survival will be very low.

Rearing tanks can be circular or rectangular FRP or concrete tanks with proper slope on the outer side for larval collection. Provision for clear filtered sea water, freshwater and aeration should be made.

Tanks in the size of 4 - 5 tonne capacity are preferable for operational convenience. Larvae can be reared in the same rearing tanks upto 25-30 days or it can be transferred/thinned to other tanks after 14 days depending upon the larval density in the rearing tank.

3. Larval Stocking Density

Freshly hatched healthy larvae (Hatchlings) from the incubation tanks are transferred carefully to the rearing tanks. Larvae are stocked initially @0 – 50 nos/litre. Depending upon the age and size, the larval density is reduced to 20 – 25 nos/l on 10th day and later and after 15 days, the density is maintained around 10 – 15 nos/l.

4. Feeding the Larvae

4.1 Live Feed

The following live feed are very important for feeding the larvae (for culture details – Refer - Live Feed culture).

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.

Whatever good the culture system may be in many cases, Rotifer or Artemia nauplii produced in the hatchery may not be having all the nutrients required for the larvae, (especially the unsaturated fatty acids), the cultured Rotifer/Artemia are enriched with nutrient rich media and then fed to the larvae.

5. Water Change

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. UV radiation treatment is also given, to get rid of the pathogenic organisms. If chlorine treated water is drawn, residual chlorine should be removed, since, fish larvae are highly sensitive to chlorine and water should be used only after de chlorination.

In the larval rearing tanks, the larvae stocked as well the live feed supplied for the larvae will excrete nitrogenous metabolites and other debris also will accumulate.

They have to be removed carefully. The debris and bottom sediment are removed by siphoning using siphon tubes. The bottom debris is slowly siphoned out along with water into a trough with filter net. The mesh size of the filter net used will be 100 – 200µfor water change upto 9 days. Afterwards filter net with 200 – 400µmesh size can be used. The water will flow through the net filter and the larvae siphoned out along with the sediment will be retained in the trough. After the required water reduction is done, the larvae along with debris are carefully transferred to another small trough. Healthy larvae are picked up and reintroduced into the tank. Dead larvae and debris are discarded. To maintain water quality in the larval rearing tanks, 30 – 40%water change is done daily. The salinity should be maintained around 30 ppt. And the desirable range of temperature is 27 – 29°C. The water level reduced (30 – 40%in the rearing tank is levelled up with filtered quality seawater and green water after taking cell count of the algae in the rearing tank.

Algal water is added daily upto 15th day. After bottom cleaning and water reduction, while water change is done, algal water is also added depending upon the concentration, (around 20 thousand cells/ ml in the rearing tank). This algal water play an important role is the larval rearing tank. Algal water added should not be contaminated since in the open culture there is chance of contamination by flagellates, ciliates and filamentous algae which will be toxic to the fish larvae. Apart from being a source of feed for the rotifers in the tank, the algae also help in the conversion of harmful excretory products like ammonia and other metabolites in the rearing container into less harmful nutrients.

4.2 Feeding

Feeding the larvae should be done with utmost care. Under feeding will lead to starvation and cannibalism in seabass larvae. Excessive feeding that too feed like rotifers will remain in the tank and excrete toxic metabolites deteriorating conditions in the tank. Feed rationing and feeding depends upon the larval density and conditions of the larvae.

Rotifer (*Brachionus plicatilis*) are given as feed to the larvae from 3rd day. Rotifer is maintained in the larval rearing tanks at concentration @0 nos./ml initially. From 4th day to 15th day the rotifer concentration is increased to 30 – 50 nos./ml gradually. And concentration is increased to 6 – 10 nos/ml from 9th to 15th day of rearing. Every day after water exchange, the food concentration in the tank should be assessed and fresh rotifers should be added to the required concentration.

In the early stages (3 – 5 days) the larvae may not be in a position to ingest the large sized rotifers. Hence after collecting the rotifers from the tanks small sized rotifer less than 100 µshould be sieved using suitable mesh size bolting cloth nets. Rotifers

collected are passed through bolting cloth net of 100 micron and the rotifers passes through are collected and fed to the early larvae. From 6th day assorted size rotifer can be given as feed.

Artemia nauplii are given as feed along with rotifers and green water from 10th day. By this time the larvae will be around 4 mm TL in size. Larvae can be feed exclusively with Artemia from 16th day to 24th day. The density of the brine shrimp nauplii in the rearing medium is maintained @000 nos./l initially and gradually increased to 6000/l as the rearing days progress. The daily ration of Artemia nauplii feeding is adjusted after assessing the unfed Artemia in the rearing tank at the time of water exchange and the larval density.

4.3 Feed density/quantity to be given to seabass Larvae at different days of rearing

Days	Feed				
Larval age	*Chlorella/ Tetraselmis/ Isochrysis* Conc. Thousand cells/ml	Rotifer (*Brachionus plicatilis*) nos/ml	Artemia nauplii Nos/L	Artemia biomass Nos/l	Cooked minced fish/shrimp meat% body wt. Per day
3 – 8	20	20	-	-	-
4 –15	20	30 - 50	2000-3000	-	-
16 –25	-	-	4000-6000	-	-
26th day onwards	-	-	-	1000-1500	30 - 40

By 21st day the larvae will be around 10 – 11 mm TL in size after completing larval development stages. From 25th day the larvae can be fed with *Artemia* sub adult (biomass) along with cooked minced fish/shrimp meat. The fry can also he weaned slowly to artificial feed.

Under circumstances, when the rotifers could not be fed with marine *Chlorella* adequately, the nutritional quality of such rotifers may be poor. In such case, the rotifers can be enriched with special enrichment media. Enrichment is done by keeping the rotifers in emulsified enrichment medium like SELCODHA or cod-liver oil for 18 - 24 hours. By this process, the animals will ingest the enrichment media which is rich in

Poly unsaturated Fatty Acids (PUFA), required for larval growth. The animals are washed and fed to the larvae. In this way Rotifers *Artemia* nauplii/*Artemia* biomass can also be enriched and fed. Moina a cladoceran can also be fed to the seabass larvae after 21 days.

6. Grading

Seabass while growing exhibits differential growth rate, hierarchy, resulting different size groups in the same rearing tank. The large one's shooters dominate others for food and space and also prey on them. Seabass larvae are highly cannibalistic and it is more pronounced in early stages. In the rearing tanks, when the larval concentration is more and congregation takes place for food and feeding, the larger ones are tempted to feed on the smaller ones. To avoid this problem, regular grading has to be done. The large sized larvae, (Shooters)' have to be removed. Uniform sized larvae should be kept in the rearing tanks for better survival and growth. Grading should be done once in three days from 15th day or whenever different size larvae are seen in the tanks. Grading can be done using a series of fish graders with different pore size of 2 mm, 4 mm, 6 mm, 8 mm, 10 mm. When the larvae are allowed to pass through the graders, different size will be retained according to pore size of the sieves. Grading may cause injuries leading to mortality. Hence proper care should be taken in handling the larvae. Prophylactic treatment with 5 ppm Acriflavin can be given.

By adopting these practices survival rate upto 48% has been achieved with average survival rate of around 15 % in 25 days in larval rearing phase. The protocols mentioned are some of the guidelines to be adopted in larval rearing. Rate of water change, feeding etc. depend upon the various environmental conditions and the conditions in the larval rearing tanks. After rearing the larvae in the hatchery for 25 – 30 days the fry can be transferred to nurseries for further growing.

7. Conclusion

The procedure and proposal mentioned are only guidelines. Situation may warrant changes in all steps to get better survival and growth. The environmental parameters like water temperature, climate conditions etc should be considered in the protocols like water changing, feeding etc. The larvae are carnivores preferring feed like zooplankton in the early stages, tend to continue with the same feeding habit even in the late phase of the life history. However, providing live fish/crustacean juveniles to the fish in the nursery rearing or grow out phase will pose practical problems on the

availability of adequate quantity. It is felt the larvae can be weaned to feed on formulated inert diet (pelletized feed) in the early stage so that, further rearing will be easily managed.

LIVE FEED CULTURE

M.Kailasam and A.R.Thirunavukkarasu

A. ALGAL CULTURE

Finfish larvae in the early stages of development are planktivorous feeding mainly on zooplankton. Adequate quantity of quality live feed of required size, that can be injested by the larvae should be made available during the larval rearing phase. The different types of phytoplankton live feed that are used as feed for rotifer in the larval rearing of seabass, *Lates calcarifer*, include green algae *Chlorella* sp, *Nannochloropsis* sp, *Isochrysis* and *Tetraselmis* sp. The culture and supply of live feed organisms have a direct bearing on the success of any larval rearing practice.

Pure cultures of the above organisms have to be continuously maintained in controlled laboratory conditions to ensure a constant source of starter culture. Live feed culture should be initiated at least two months prior to the spawning season, to provide a continuous supply of it for larval rearing.

MICRO ALGAE

Micro algae are the plant component of plankton. They are unicellular and microscopic in size. They are the primary producers of organic matter via photosynthesis. Since, micro-algae are the biological starting point of the energy flow through the most important aquatic food chain, the grazing food chain, it is logical that management of algal production is an integral part of many hatcheries operational. Micro-algae not only play an important part as a food source, but, together bacteria, they also have an important role in the oxygen and carbon dioxide balance in the cultures. Recently more than 40 different species of micro-algae, isolated in different parts of the world, are being used in intensive culture procedures. The most frequently used species are *Nannochloropsis spp, Chlorella spp, Isochrysis galbana, Isochrysis tahiti, Monochrysis lutheri, Tetraselmis suecica, Dunaliella* spp and the *chlorococcalean* (*Chlorella* spp).

Criteria to be considered in the selection of micro-algae

1. Size and feeding density
2. Motility and floating capacity
3. Nutritive value
4. The digestibility and absorptive. High nutritive value with poor digestibility is not desirable
5. Reproducibility – It should be possible to produce in large quantities
6. Cost should be low.

CHLORELLA CULTURE

Propagation and Maintenance of Stock Culture of *Chlorella spp/Nannochloropsis spp.*

Preparation of Culture Medium (Convey's medium).

Stock solutions of different inorganic nutrients, trace elements and EDTA are to be prepared by dissolving each compound separately (Table 1) in 1000 ml distilled water.

Table 1. Culture medium for *Chlorella Spp.* :

Chemicals	Quantity (g/l)
Potassium nitrate	202.0
Sodium dihydrogen phosphate	310.0
Sodium monohydrogen phosphate	89.0
Calcium chloride	14.7
Trace elements	*
Fe.EDTA Complex	*

To prepare one litre working solution of *Chlorella*, from the stock solution of each compound, 1 ml is added to 1 l of sterile seawater. Potassium nitrate and sodium dihydrogen phosphate are to be added at the rate of 5 ml and 2 ml respectively. Adjust pH to 7.5 – 8.0.

- Dissolve 61 mg boric acid, 169 mg Manganous sulphate, 287 mg zinc sulphate, 2.5 mg copper sulphate and 12.36 mg Ammonium molybdate in 1000 ml of distilled water.

- Dissolve 6.9 g ferrous sulphate and 9.3 g of disodium salt of EDTA in 800 ml distilled water. Bring it to boil, cool it and make up to 1000 ml by adding distilled water.

For a pure culture of *Chlorella spp.*, propagation is to be initiated by inoculating on agar slants. Agar slants are prepared in rimless Borosil/corning test tubes (20 ml) using *Chlorella* medium to which 1.5% agar is added and autoclaved. The slants are inoculated with *Chlorella* using a platinum loop and cotton plugged. Transfer these slants to axenic conditions at 20-25°C with continuous fluorescent illumination (1000-15000 lux). A multi step culture procedure can be followed for further sub-culturing and maintenance

of the culture. Here, the cells from agar slants are transferred to liquid culture medium in 20 ml test tubes, then to 100 ml conical flasks, 250 ml conical flasks and upto 1 l flasks (10-20% of inoculum is used in each step). The flasks are shaken at regular intervals and allowed to grow for 7-10 days.

Growth Characteristics:

The population of algae is characterized by a sigmoid curve and is divided into for distinct phases.

1. The Lag phase – characterized by zero growth. The population remains unchanged. The newly added inoculum adopts to culture condition.

2. Logarithmic or exponential phase: The cells in this phase divide fast in constant geometric progression. Cells have active metabolic rate.

3. Stationary phase: Population remains constant or steady. This may be caused by nutrient limited medium and aging of cells.

4. Death Phase: This is the phase of declining growth. Usually algal culture collapse and nutrient in the medium is already exhausted.

Physical requirements for the culture of algae

a. Illumination

The absence of sunlight in "controlled rooms" a/c provided by cool – white day light fluorescent tubes.

b. **Temperature**

c. **Aeration:**

Aeration is provided to keep the algae in suspension, to partly supply carbon needed for plant growth, disperse dissolve materials and to avoid adherence of cells to the walls of culture vessels.

Different methods for stock cultures:

1. Serial dilution
2. Repeated sub-cultures
3. Capillary pipette method
4. Streak plating

Estimation of Chlorella Density

Materials
Binocular microscope
Haemocytometer
Cover slip for haem cytometer-0.4mm thick
Pasteur pipettes
Fixative-Lugols Iodine
30% ethanol

Step 1

Collect a sample of the phytoplankton from a well mixed culture (10-20ml). Fix with 1-2 drops of Lugols solution, within 15mts of collection.

Step 2

Clean the surfaces of the haemocytometer and cover slip with 30% ethanol and wipe dry. Place cover slip on the counting area.

Step 3

Mix sample well. Draw sample into the pipette and place the tip of the pipette near the V-shaped notch of the haemocytometer. Allow the sample to flow smoothly and evenly into the entire chamber. With a second sample load the second chamber. Wait for 10mts prior to counting.

Step 4 (When cell density is 1-25 x 10^6 cells / ml)

While viewing through the microscope count the number of cells in the four corner squares of the haemocytometer each of which is sub-divided into 16 squares. Repeat the process on the other side of the haemocytometer. The result will be 8 separate counts. Using the following formula the average number of cells per square will be obtained.

$$\text{Average \# of cells counted} = \frac{\text{Total \# of cells counted}}{8}$$

$$\text{Estimated cell density} = A \times 25 \times 10^4 \text{ cells/ ml}$$

$$= \frac{A \times 25 \times 10^6}{100} \text{ cells / ml}$$

Greater than 25 x 10^6 cells /ml

View the central square of haemocytometer through the microscope. The central square is divided into 25 smaller square, and each of the 25 squares is further sub-divided into 16 sub-squares. Count the number of cells in 5 of the 25 squares. Five such squares are counted on each side of the haemocytometer, ie 10 per sample. The cell density of the sample can be estimated by the following fomula.

$$\text{Average \#of cells counted} = \frac{\text{Total of cells counted}}{10}$$

$$\text{Estimated cell density} = A \times 25 \times 10^4 \text{ cells/ ml}$$

$$= \frac{A \times 25}{100} \times 10^6 \text{ cells/ ml}$$

Mass Culture of *Nannochloropsis*

Out door mass production of *Nannochloropsis* spp consists of two stages. In the first stage, 500 l tanks are inoculated with starter cultures and in stage two, tanks of greater capacity are inoculated from cultures drawn from the 500 l tanks, after they attain harvest stage. (30 – 40 x 10^6 cells/ml).

The 500 l tanks are cleaned well and filled with filtered sea water. The seawater chlorinated with 5.25%ommercial bleaching powder solution @000 ml/1000 l. Vigorous aeration is provided for 24 hours and subsequently dechlorinated with 150 ppm of sodium thiosulphate solution. Ensure that all chlorine is removed, then add a nutrient mixture of Ammonium sulphate, single Super Phosphate and Urea in the ratio 100_g:10_g:10_g per tonne of sea water. Add *Nannochloropsis* inoculum derived from the stock culture laboratory. The initial stocking density should be around 3 – 5 x 10^6 cells/ml. Vigorous aeration and bright sunlight are essential for cell multiplication. This culture is in turn used to inoculate *Nannochloropsis* spp in larger tanks, using 1/5 *Nannochloropsis* spp starter volume and 4/5 filtered seawater. Some nutrient mixture is added in these tanks also. Continuous aeration and sun light provides good cell density. When 20 – 25 x 10^6 cells/ml is reached, it can be used to feed rotifers and as green water for larval rearing tanks.

Conclusion

The algae being an important component as feed for both the zooplankton and also for conditioning rearing medium, it should be maintained as pure and healthy as possible. Any continuous or crashing of algae cultives will have chain effect in the hatchery operation of seabass.

Using a fine dropper, individual specimens are isolated and introduced into a glass cavity block containing filtered, sterilized sea water, the pH of which is adjusted to be same as that of the field sample.

Adjust the pH of *Nannochloropsis* spp to 7.5-8.0 using dilute Sulphuric acid or Hydrochloric acid or if needed with Sodium hydroxide solution. *Nannochloropsis* spp may also, be centrifuged at 3000ppm for 10mts and resuspended in sea water. Estimate the density of *Chlorella* spp using a haemocytometer. Prepare *Nannochloropsis* spp density to about one million cells/ml, by adding appropriate quantity of filtered sea water.

Distribute the *Nannochloropsis* spp into 5-10 ml glass cavity blocks. Using a Pasteur pipette, transfer each of the rotifer species into the cavity blocks. Serially transfer the isolated individuals through several cavity blocks to eliminate any associate organisms. Cover the cavity block and place in diffused light.

After isolation, replace with fresh *Nannochloropsis* (density 1 million cells per ml), every 12 hrs. Transfer adult rotifer along with eggs and neonates if anyt. Gradually increase the volume to 25ml, in 50ml beakers. Change the culture daily once. Use 50-80 um mesh to separate the rotifers. Proceed till the density reaches about 50 individuals/ml and the volume up to 500ml. Increase *Nannochloropsis* spp density to 3-4 million cells/ml.

When the density exceeds the above, remove half the quantity and make up clean water. Change the culture daily with fresh *Nannochloropsis* spp at the above food density.

Incubation temperature for algal cultivation often ranges between 18°C – 25°C. The scaling up of outside culture is usually done early in the morning to avoid temperature shock.

B. Rotifer (*Brachionus plicatilis*) Culture

Importance of Rotifer

Rotifers are microscopic organisms abundantly found in all the aquatic systems. It thrives in the eutropic condition. Rotifer succeeds normally after the phytoplankton bloom crashed out. It feeds on macroscopic unicellular algae like *Chlorella*, *Tetrselmis*, *Nannochloropsis* etc., Because of their apt size (100 -280µ) which may fish larvae can ingest, it is cultured in large scale and used in all the fish hatcheries. Rotifer is considered as an important live food organism.

Types of Rotifers:

There are three types of rotifers cultured in the hatcheries and used depending upon the requirements.

1.	SS (super small) type	-	100 - 140µm
2.	S type	-	141 - 220µm
3.	L type	-	Above 221µm

Generally the water volume of algal culture is 2-5 times greater than the volume of rotifer culture. The required daily parameters for rotifer culture are densities of *Nannochloropsis* spp > $10X10^6$ cells/ml to be provided for the rotifers, temperature range between 27-28°C. Rotifer starter cultures are drawn from stock cultures maintained. Fiberglass tanks varying in capacity from 1-2 ton are used for rotifer culture. Tanks should be preferably elevated at about 3 ft above the ground for easier collection and harvest.

Propagation and Maintenance of Pure Culture of Rotifer

To raise a pure culture of rotifer, initial samples must be collected from stagnant water bodies, using a net of 50-80µ mesh size. About 50-60 l of pond water then filtered, yields sufficiently large numbers of individuals of *Brachionus plicatilis*.

Using a fine dropper, individual specimens are isolated and introduced into a glass cavity block containing filtered, sterilized seawater, the pH of which is adjusted to be same as that of the field sample.

Adjust the pH of *Nannochloropsis* spp to 7.5 – 8.0 using diluted Sulphuric acid to Hydrochloric acid if needed with Sodium hydroxide solution. *Nannochloropsis* spp may also be centrifuged at 3000 rpm for 10 minutes and resuspended in seawater. Estimate the density of *Nannochloropsis* spp using a haemocytometer. Prepare *Nannochloropsis* density to about one million cells/ml by adding appropriate quantity of filtered seawater.

Distribute the *Nannochloropsis* spp into 5-10 ml glass cavity blocks. Using a pasteur pipette, transfer each of the rotifer species into the cavity blocks. Serially transfer the isolated individuals through several cavity blocks to eliminate many associate organism. Cover the cavity block and place in diffused light.

After isolation, replace with fresh *Nannochloropsis* (density 1 million cells per ml) every 12 hours. Transfer adult rotifer along with eggs and neonates if any. Gradually increase the volume to 25 ml, in 50 ml beakers. Change the culture daily once. Use 50-80µ mesh to separate the rotifers. Proceed till the density reaches about 50 individuals/ml and the volume up to 500 ml. Increase *Nannochloropsis* density to 3-4 million cells/ml.

When the density exceeds the above, remove half the quantity and make up with clean water. Change the culture daily with fresh *Nannochloropsis* at the above food density.

Mass Culture of Rotifer

Clean the rotifer culture tanks add *Nannochloropsis* water at a density >$20X10^6$ cells/ml are added. Inoculate the tank with rotifers to achieve an initial density of 10 ind/ml. Estimate density by taking 1 ml aliquot with a glass rotifer pipette and count the number of rotifers in the pipette with a 10X magnifier. Allow 7-8 days for rotifer density to increase. Harvest and concentrate using a 48µ plankton net. Reserve some

harvested stock as starter culture for other tanks and rest as feed for larvae. After each harvest, thoroughly wash and clean tanks with fresh water.

Culture of rotifer and algae should be scheduled to ensure daily harvest of rotifer and an uninterrupted production. Culturists prefer rotifers to reproduce asexually, because of the shorter life span and better nutritive value of the asexual forms. This is accomplished by regulating feed, water, temperature, salinity and aeration during the culture process. Fertility is a measure of the general health of the rotifer culture. Under normal circumstances greater than 30% of the rotifers should be carrying eggs 24 hrs after initial stocking. This value will fall to 10% at the time of harvesting.

C. ARTEMIA NAUPLII AND BIOMASS

Artemia

Brine shrimp, Artemia is an important source of animal protein for the fast growing larvae of seabass. Artemia is rich in required PUFA and aminoacids needed for early stage of the larvae.

Biology of *Artemia*

Artemia culture in the hatchery is starts with cysts. Cysts are metabolically inactive encysted embryos. Dry cyst measures 232-240 µm in diameter and weight 3.70 µg. On an average 1 gm cyst contains about 2,65,957 ball live particles, which after immersion in normal seawater hatches into *nauplii*. After hydration of cyst measures 237.4 ±6.60 um in diameter and weigh 3.70 µg. *Nauplii* at the first stage is known as INSTARI. It metamorphoses upto INSTAR XII when the *Artemia* attains adult stage.

Adult *Artemia*

In about 12 days, the individual attains the length of 7.5 mm. Sexual dimorphism is pronounced by this time. The male antennae get transformed into hoodlike muscular graspers, whereas in the posterior part of the trunk region, a paired copulatory organ can be observed. Female *Artemia* can easily be recognized by the broodpouch which is situated just behind the 11th pair of thoracopods. The individual become gravid by 15th day, carrying a broodsac full of eggs. The total length of *Artemia* at this stage is about 9-10mm.

Reproduction

Two different patterns of reproduction viz, oviparous and ovoviviparous have been recorded in *Artemia* under different feeding and environmental regimes. Organisms cultured in low salinity of 35-60 ppt with high dissolved oxygen, 94.7-7ppm), pH levels 8.2 – 9.2 are conducive for ovoviviparous reproduction.

Oviparous reproduction was reported to occur in high salinity and low oxygen levels. In oviparous reproduction, eggs develop into the gastrula stage, become surrounded by the thick shell and are deposited as cysts. In hypersalinity conditions, cysts are released, which float in the brine water. The floating cysts are eventually blown ashore, where they accumulate in large masses and dry.

Production of *Artemia* Nauplii

The production of *nauplii* by incubation of cyst in seawater is a very simple procedure. However when working on a large scale and with high densities of cysts, several parameters might be critical in ensuring maximum hatching efficiency.

Optimum condition for hatching

Temperature	-	25-30°C
pH	-	7.5 – 8.5
Salinity	-	26 ppt
Oxygen	-	above 2 ml/L
Illumination	-	above 1000 lux
Cyst density	-	1 gm/liter

Hatching

Best hatching efficiency with high densities of cysts can be achieved with transparent funnel shaped containers that are aerated from the bottom. The hatching containers are about 20-30 liter capacity, cylindroconical FRP tanks. The hatching tanks are illuminated at a distance of about 20 cm with 60 watt fluorescent lamp. A continuous aeration from the bottom of the hatching tank ensure that all cysts are kept in suspension. Complete hatching takes place within 24-36 hrs. When hatching is complete using light source, the nauplii can be attracted and collected by siphoning.

Decapsulation of cyst

The hard dark brown external layer of cyst, the chorion can be removed without effecting the viability of the embryo by short term exposure of the hydrated cyst to a hypochlorite solution. Decapsulation improves the hatching efficiency.

Decapsulation Procedure

- Hydrate cysts in seawater for atleast 1 hour.
- Preparation of decapsulation solution

  Decapsulation solution with bleaching powder
  - $Ca(OCL)_2$: 0.5g active product/g of cyst
  - Na_2Co_3: 0.78 g/g of cyst.

Aerate this solution for 24 hrs. After 24 hrs stop aeration and allow to sedimentate. Only use the upper part of decapsulation solution. Decapsulation solution – $Ca(OCL)_2 + Na_2Co_3 + H_2O$ =3.3 ml/g cyst.

- Drain out the hydrated cyst into fine mesh sieve
- Put the hydrated cyst into decapsulation container, add decapsulation solution and aerate
- Wait till cyst become orange in colour
- Drain the suspension of decapsulated cyst into a fine mesh sieve and rinse immediately, until the smell of hypochlorite is removed.
- Soak and stir in O.IN HCL solution for less than 1 minute
- Wash
- Soak and stir in 0.1% $Na_2S_2O_3$ for less than 1 minute
- Wash
- Incubate for hatching

Biomass Production

As the seabass larvae grow, the feed requirement and the preying tendency of the fry increases. Adult Artemia biomass meets this requirement of the seabass fry.

Biomass production under controlled condition can be carried out either in batch or in flow through culture systems. In both the culture systems, provision are made to maximize oxygenation of the medium and to ensure food availability to all the larvae, when culturing in high density.

Batch Culture System

In batch culture system nauplii are reared upto adult stage, without any water renewal in airlift raceways. Freshly hatched nauplii are stocked at a rate of 10,000/liter and feeding is done with rice bran and water transparency is maintained at 15-20 cm. As *Artemia* is a nonselective filter feeder, it can be cultured by feeding with a wide range of feed like *Chaetoceros*, *Skeletonema*, marine algae and yeast etc. Adequate food must be available in the medium at all times as Artemia is a continuous filter feeder. Faecal pellets and excreta have to be removed regularly from the culture medium from 4th day of culture, as they affect water quality. pH of the water should be maintained above 7.5. Harvesting is done with a scoop net.

Flow Through Culture System

More intensive artemia culture can be achieved with flow through system in which continuous renewal of culture water will be maintained but in all other aspects it resembles the batch culture system. Continuous inflow of fresh culure medium with food to the culture tank is maintained. The continuous water change results in removal of all metabolites and hence *Artemia* culture can be carried out with intensive i.e. 20,000/litre.

OXIDATIVE STRESS AND ITS IMPACT ON QUALITY.SEED PRODUCTION

N. Kalaimani

IMPLICATIONS OF OXIDATIVE STRESS

Oxidative stress has been implied, in the case of diseases that include immune injury, drug and toxin induced reactions, ischaemia and subsequent reperfusion injury, nutritional deficiencies, radiation injury, aging, hemolytic diseases, lung disorders, heart and cardiovascular system, kidney and gastro intestinal disorders and diseases affecting the brain, nervous system, neuromuscular disorders, cataract and retinal damages and a variety of skin diseases. In most diseases and as well as in several conditions of toxicity, increased oxidant and free radical formation is a consequence of the disease. Many pathological conditions have been implicated as resulting from damage caused by active oxygen/free radicals (oxy-rad) such as superoxide, hydrogen peroxide, hydroxyl radical, lipid radicals and nitrogen oxide.

FREE RADICALS, REACTIVE OXYGEN SPECIES IN CELL AND TISSUE DAMAGE

Molecular oxygen takes up electrons during respiration in the living cells in a sequential and orderly manner. Intermediates produced during this process are oxidants like superoxide and hydroxyl radical and hydrogen peroxide. (Saugstad, 1989).

$O_2 + e^- \longrightarrow O_2^-$ (Superoxide anion)

$O_2 + e^- + 2H^+ \longrightarrow H_2O_2$ (Hydrogen Peroxide)

$H_2O_2 + e^- \longrightarrow OH^- + OH^\bullet$ (Hydroxyl radical)

$OH^- + H^+ \longrightarrow H_2O$ (Water)

Superoxide and hydroxyl free radicals together with H_2O_2 and singlet oxygen are called the Reactive Oxygen Species (ROS) or peroxidants, and are endogenous in origin. They interact with exogenous free radical inducers and with nitric oxide (NO). Oxygen radicals are formed (*albeit* in small amounts) during cellular respiration and may leak out of the mitochondria and interact with endoplasmic reticulum, plasma, membranes and other structures. A second source of ROS is the xanthine oxidase enzyme which also catalyses the univalent oxidation of purines with concomitant formation of superoxide radical, H_2O_2 and perhaps singlet oxygen .

Another source of ROS is the activated neutrophils, undergoing 'respiratory burst' as a sequel to the detection of foreign body in the system. Other sources of FR generation are by the action of epinephrine, prostaglandin synthesis and calcium overload. Exogenous agents to produce active oxygen include photochemical smog and anticancer drugs. Added to this, transition metals, iron and copper contained in our body promote the generation of the most highly reactive class of active oxygen known as hydroxyl radical. With the discovery of the biology of NO, the role of oxidants in cell damage has become better understood more than ever since most cells can produce not one but two radicals, O_2^- and NO. When NO and O_2^- coexist, these can react to give $ONOO^-$, a potent oxidant. It has been shown that $ONOO^-$ rather than O_2^- or NO is the most likely candidate for the actual cytotoxic molecule in reperfusion injury.

Oxidants are also encountered in the living cells formed by the nitroso derivatives of proteins or amino compounds. Nitrates and nitrites present in food and water can react to form nitroso proteins in the stomach and other parts of alimentary tract. The nitroso group is a free radical inducer. Formation of Nitrates and nitrites due to environmental pollution by residual feed may result in the formation of nitroso compounds which induce free radical formation which affects the health of the animal.

The metal chelating agents such as transferrin, lactoferrin and ceruloplasmin to bind harmful metal ions are also present to minimise the detrimental effects of oxy-rad. Cellular damage occurs in the condition in which the rate of oxygen radicals (oxy-rad) formation is increased and/or the activity of the defense system is impaired. Oxidatively damaged cell constituents can be removed and repaired by a restoration system that the cells possess. The pathways for the generation of reactive oxygen species and of the actions of some of the enzymes involved in antioxidant defenses in the cell. Is depicted in Figure 1.

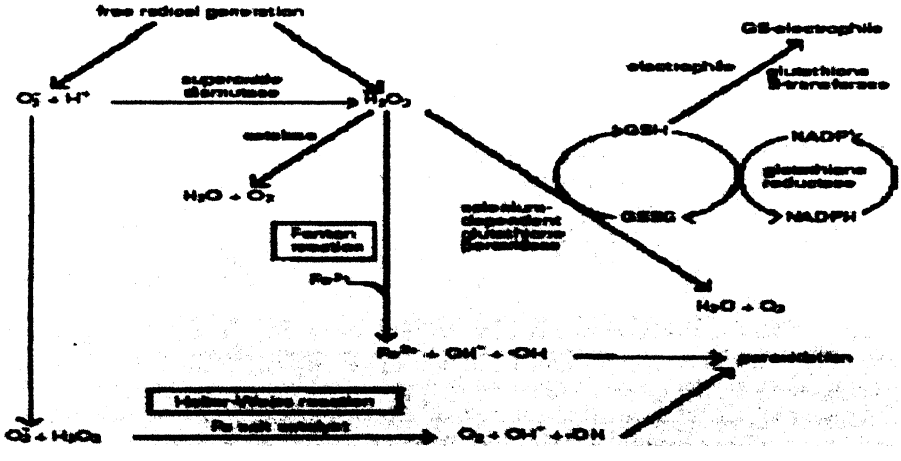

Figure 1 - Summary of the pathways for the generation of reactive oxygen species and ofthe actions of some of the enzymes involved in antioxidant defenses in the cell. Storey, K B.,(1996)

Prevention and control of diseases using antioxidants:

Successful prevention or control of some of the diseases and toxicities using antioxidants and free radical scavengers has confirmed the role of free radicals and oxidants in toxicology and diseases. This is further confirmed from a variety of reports in which free radical scavengers and antioxidant enzymes are able to prevent or partially inhibit the pathological changes. Experimental production of colorectal cancer in mice was inhibited with Vitamin E (a free radical scavenger) in the diet. This was one of the earliest findings in this direction.

Many potent chemical carcinogens are metabolised into free radicals and potentiated the generation of Superoxide and Hydroperoxide In studies made with naphthyl amines and azodyes, it has bee demonstrated that a correlation existed between carcinogenesis and the formation of free radicals and H_2O_2. Aflatoxin B_1 is also found to have the toxic function by the generation of free radicals and H_2O_2 AFB_1 induced killing of rat hepatocytes can be countered successfully by catalase and SOD (which destroy H_2O_2 and Superoxide radicals respectively), and mannitol, deferoxamine and other free radical scavengers. Most of the known chemical carcinogens are capable of forming free radicals in the host cells and also react with endogenous H_2O_2 to form superoxide and or hydroxyl radicals.

MECHANISM OF DAMAGE TO CELLULAR ARCHITECTURE BY FREE RADICALS

Reaction with lipids

Free radicals attack at various levels in the cellular architecture and a few of these are described below. They damage cells through lipid peroxidation (LPO) on membrane producing structural and functional changes in the cell membrane. Membranes are dynamic fluid structures where lipids and proteins are held together and the fluidity is closely related to the presence of polyunsaturated fatty acids (PUFA) side chains. Oxidative deterioration of PUFA is triggered by an abstraction of hydrogen either by free radicals or ROS and the lipid peroxidation is different from the cycloxygenase action. The reaction is outlined below:

$$\text{Lipid -H} + R^{\bullet} \rightarrow RH + \text{Lipid}^{\bullet} \text{ ($R^{\bullet}$ being the free radical)}$$

Molecular rearrangement on the fatty acyl chain forms a conjugated diene which takes up a molecule of oxygen.

$$\text{Lipid –H} + R^{\bullet} \rightarrow RH + \text{Lipid}^{\bullet} \text{ (Hydrogen abstraction step)}$$

$$\text{Lipid}^{\bullet} + O_2 \rightarrow \text{Lipid OO}^{\bullet} \text{ (lipid peroxide radical)}$$

This free radical (Lipid $OO^{\bullet}$) can abstract hydrogen from another molecule of lipid to form lipid hydroperoxide .

$$\text{Lipid } OO^{\bullet} + \text{Lipid} - H \rightarrow \text{Lipid } OOH + \text{Lipid}^{\bullet}$$

and the chain reaction continues.

Lipid hydroperoxide can also be formed by the action of singlet oxygen on a lipid.

$$LH + {}^{1}O_2 \rightarrow L{\bullet}OOH$$

Lipid hydroperoxides are unstable and breakdown in biological systems giving rise to a variety of compounds including malondialdehyde (MDA) and 4-Hydroxy nonenal (HNE). Smaller components of LPO are 2-alkenals, and proteins and phospholipid bound aldehydes which may also be toxic. LPO products are measured by their reaction with thiobarbituric acid, and have been generally used as an index of oxidant and free radical damage.

Lipid peroxidatiom

Lipid peroxidation, (LPO) in biological membranes causes alterations in fluidity, fall in membrane potential, increased permeability to H^{+} and other ions and eventual rupture leading to release of cell and organelle contents such as lysosomal hydrolytic enzymes. Earlier it was shown that the disrupted or damaged tissues undergo lipid peroxidation at a faster rate than their healthy counterparts. Hence the sequence of events may be conjured up as Disease or Toxin → cell damage or death → increased LPO

which will well explain the elevated lipid peroxidation products in disease and toxicology.

Reaction with proteins

Free radicals and oxidants react with proteins mainly in two ways. By abstracting hydrogen from thiol groups, proteins are oxidised, leading to disulphide linkages and the resultant conformational and functional changes. Such reactions occur in the cells due to the simultaneous presence of molecular oxygen, transition metal ions of iron and copper and free radicals or free radical inducers. These are named 'Mixed Function Oxidation' and have been renamed 'Metal Catalysed Oxidation' (Levine *et al.*, 1990). Oxidative stress can result from exogenous sources i.e., red-ox active xenobiotics or free radical generators. The possible fate of oxidised cellular proteins can be depicted as,

Proteins → Denatured/Hydrophobic proteins

This leads to cross-linking and formation of insoluble aggregates, fragmentation and increased susceptibility to proteolysis. Denaturation occurs due to variety of possibilities such as

(a) The interconversion of aminoacids, exemplified by the oxidation of cysteine (SH) to cystine(-S-S-).

(b) Other alterations in the aminoacid side chain reported include Carbonyl oxidation of the lysine residue forming γ glutamyl semialdehyde and

(c) hydroxylation of phenylalanine to tyrosine.

These alterations change the primary structure of the proteins, the isoelectric point, folding and hydrophobicity. Hydrophobic patches on proteins contribute to protein aggregation.

Reaction with DNA

Exposure to free radicals and oxidants and increased cellular generation of superoxide and hydroxide radicals and H_2O_2 lead to DNA strand breakage. The strand break may occur due to activation of some specific DNA-cleaving mechanism. Both purine and pyrimidine bases are modified by free radicals, especially the hydroxy radicals.

Exposure of *E.coli* cells to H_2O_2 at concentrations in the range of 1-3mM is found to be mediated through superoxide and hydroxyl radicals, which leads to DNA damage. This can be blocked by iron chelating agents which prevent the Fenton–reaction within the cells. It has been shown that possibly some cancers may originate as a result of faulty repair of DNA damage produced by free radicals.

CELLULAR DEFENSE BY ANTIOXIDANT ENZYMES AND FREE RADICAL SCAVENGERS

Avoidance of free radical formation

Potentially injurious effects of oxidants and free radicals on the living organism are prevented by a well-organised defense system and they function at four levels. The first and best effort is avoidance. This is achieved by cytochrome oxidase and other metallo enzymes. They help the cells in carrying out the tetravalent reduction of oxygen to water without releasing the toxic intermediates in a free state. Further, metal ions, which could participate in the oxidant producing reactions, are generally carried or sequestered by proteins like transferrin and ferritin, so as to minimise the amount of free iron in the cells. Similarly copper is bound to ceruloplasmin and the ionic form is not generally available in the free state. Albumin binds copper tightly and iron weakly. Haptoglobin/hemopexin binds free hemoglobin/heme. Hemoglobin and methemoglobin are powerful peroxides and can accelerate lipid peroxidation while haptoglobin inhibits the reaction by binding to haemoglobin. The antioxidant role of urate is due to its ability to tightly bind iron and copper ions.

Prevention of free radicals (FRs) acting on the cell

The second line of defense is prevention, by providing a continuous supply of GSH. The oxidised and reduced glutathiones are interconvertible and the cell maintains the bulk in the active (reduced) form. This reaction is catalysed by the enzyme glutathione reductase.

Glutathione

$$GSSG + NADPH \xrightarrow[\text{reductase}]{} 2GSH + NADP$$

The liver is the major site of GSH synthesis in humans and animals. In the liver, it detoxifies endogenous metabolic peroxides through glutathione peroxidase and of exogenous substances such as drugs and other xenobiotics through glutathione-S-transferase. GSH synthesised in the hepatic cells is either translocated to plasma or excreted into the bile through carrier mediated transport. During infection and inflammatory processes GSH is mobilised from the liver to the pathological site. Decrease of hepatic thiols is due to increased efflux of glutathione in shock induced inflammatory reaction.

Regulation of cellular levels of GSH can be broadly divided into four areas (1) Uptake of precursor aminoacids and intact GSH, (2) the regulation of the enzymes necessary for GSH synthesis, (3) alteration in the cellular redox system due to increased lipid peroxidation and cross-linking of glutathione with aldehyde, (4) direct oxidation of glutathione by ROS. Uptake of cysteine is the rate-limiting step for GSH synthesis.

GSH also forms conjugated products with ingested toxins and provide a detoxifying step, the GSH conjugates show less toxicity and are easily excreted in the faeces and urine. Glutathione-S-transferase (GSTs) are inducible and have been found to be induced in rat liver when AFB_1 is ingested.

Combating free radicals and their inactivation

The third line of defense is damage control which is achieved by providing free radical scavengers and antioxidant enzymes. The former arrests the initiation and propagation of the free radical chain reactions. They react rapidly with the free radicals, inactivate them and control the damage. While doing so the scavengers themselves are converted to radicals, which are many times less toxic than the original free radical. Vitamin E (α - tocopherol), Vitamin C (ascorbic acid) Vitamin A, β - carotene and reduced glutathione are free radical scavengers and are considered as antioxidants. These free radical scavengers interact as synergists and such interaction takes place at different levels, as detailed below :-

a Antioxidant regeneration – e.g. Vitamin E is regenerated by Vitamin C

b Protective mechanism – e.g. Vitamin E protects β-carotene from autooxidation

c Compensatory mechanism – e.g. Vitamin E ameliorates selenium deficiency and *vice versa*

d. Complementary mechanism – e.g. β-Carotene may complement Vitamin E and prevent lipid peroxidation

AFB_1 induced mutagenesis in *Salmonella* is partially inhibited by the free radical scavengers, vitamin analogues, Ascorbic acid and vitamin E. Vitamin E was more potent than vitamin C in AFB_1 induced mutagenesis. Dietary selenium supplementation was found to provide protection against AFB_1 induced neoplastic foci in rat liver.

Antioxidant enzyme activities

Antioxidant enzymes are universally distributed in all the cells and combat ROS and destroy them to prevent their interaction with cellular compounds. Superoxide dismutase (SOD) protects cells and tissues from inflammatory damage by inactivating the superoxide free radical.

$$2O_2^{-\bullet} \xrightarrow[2H^+]{SOD} H_2O_2$$

Catalase and glutathione peroxidase (GP_X) act upon H_2O_2 and inactivate it. Both the enzymes have been detected in all human cells and organs with the highest activity in erythrocytes, liver and kidney.

$$2H_2O_2 \xrightarrow[GPx]{Catalase} 2H_2O + O_2$$

$$H_2O_2 + 2GSH \longrightarrow 2H_2O + GSSG \text{ (Oxidised glutathione)}$$

GP_X is a selenium containing protein and can act not only on hydrogen peroxide but also on lipid hydroperoxide and arrest lipid peroxidation process.

Repair of free radical induced damage

The fourth and final line of defense is to repair the damage which has occurred to the macromolecules in the cell structure, function and viability of the cells. Repairs are done presumably by the accelerated removal of damaged molecules. For e.g. lipid peroxidative product in the membrane are removed by the action of specific phospholipases, followed by replacement with new molecules. Damaged proteins are degraded into smaller fragments by proteases and damaged parts of the DNA are cleared and new segments formed at the site by DNA polymerase-I.

Inefficiency at any of the four levels of defense against free radicals and oxidants may lead to susceptibility to tissue damage, manifesting as infections, inflammatory or degenerative diseases. The process is schematically represented in figure 2.

Antioxidant status in embryonic, post-hatch and larval stages of Asian seabass (*Lates calcarifer*)

The antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and selenium-dependent glutathione peroxidase (SeGPx) and low molecular weight free radical scavengers such as reduced glutathione (GSH) and ascorbic acid (Vitamin C) were evaluated during the period from gastrulation (GS) to 25 days post-hatch (dph) in the larvae of Asian Seabass, *Lates calcarifer*. Oxidative damage due to lipid peroxidation (LPO) was also assessed, by evaluating the formation of malondialdehyde (MDA).

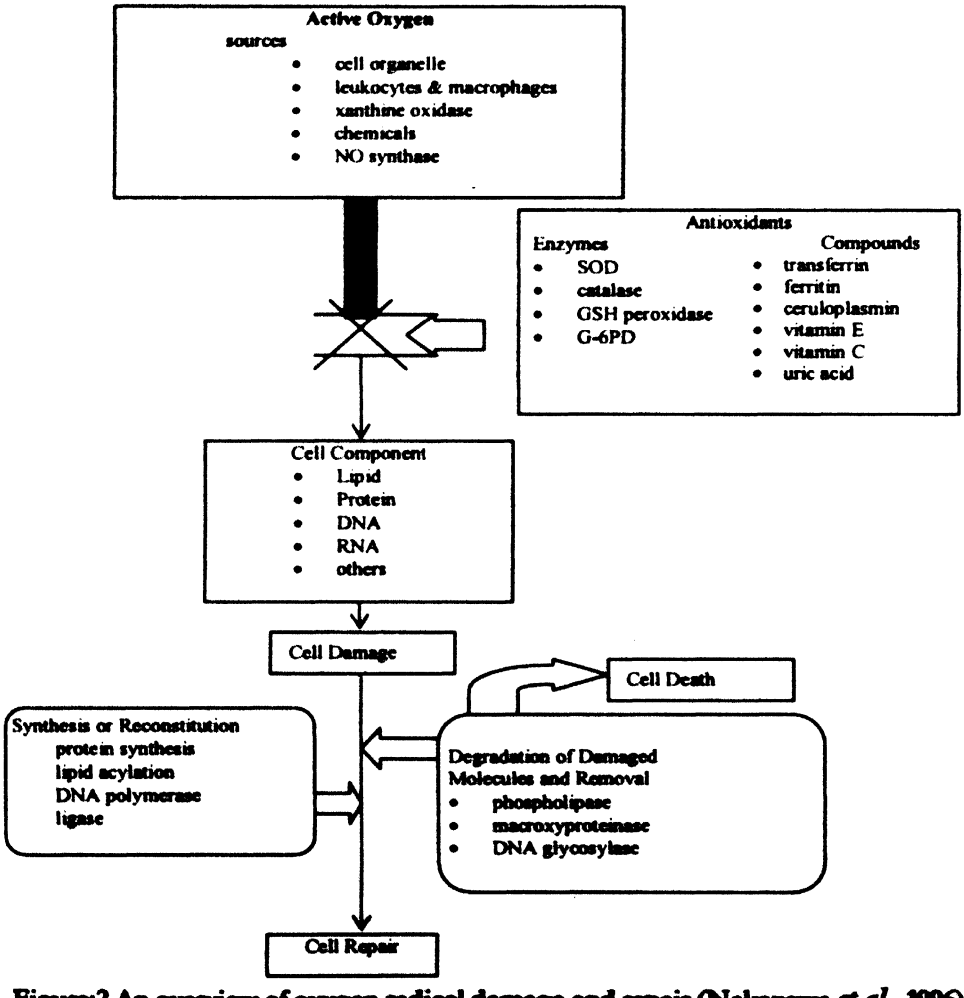

Figure:2 An overview of oxygen radical damage and repair (Nakazawa *et al* .,1996)

All the three antioxidant enzymes, SOD, CAT and GPx showed high activities during gastrulation, suggesting an increased metabolic rate during the period of embryonic

development. Though the SOD activity apparently decreased progressively during 3 dph to 20 dph of larval development, the difference was not significant. CAT showed high activity during gastrulation and remained constant up to 3 dph, suggesting an increased need to metabolize hydrogen peroxide (H_2O_2) and organic peroxides.

In contrast selenium dependent glutathione peroxidase (SeGPx) activity increased progressively from 5 dph to 25 dph during larval development indicating an increased need to detoxify lipid peroxides. This is evident from the observation of increased lipid peroxidation from 10 dph to 25 dph during larval development. GSH levels were low at gastrulation, indicating increased metabolic rate and formation of lipid radicals during this period corresponding to the decrease in ascorbic acid level, which is consumed for regeneration of GSH. The antioxidant enzyme SeGPx and low molecular weight free radical scavengers GSH and ascorbic acid and lipid peroxidation showed significant variation in their levels during 3 dph when the larvae changed their feeding from endogenous to exogenous mode.

This has been proved by the fact that supplementation with natural antioxidants such as ascorbic acid (VitC) has been found to enhance the survival rates of fish larvae giving an indication that antioxidants prevent the oxidative stress during developmental changes in various life stages of fish larvae. Young developing fish larvae and fry need vitamin C for the normal development of their cartilagenous tissues and skeleton. It has been found that pour supply of the vitamin to the young fish in hatcheries provoke most of the known skeletal deformities. Stress related responses are weakened and the fish are prone to disease. Vitamin C plays a vital role in the physiological mechanisms of stress and immune responses of the nursery and caged on-growing fish. Supplementation of the fish diets with extra vit. C at intervals is recommended in order to strengthen their defences against secondary infections and their endurance to adverse weather, handling and transportation stress. On cage farms, vitamin C supplemented diets help to speed surface wound healing in case of scale loss subsequent to fish grading or vaccination. In addition, supplementary vitamin C is required by fish suffering bacterial, viral or parasitic infections enhancing their defence mechanisms against disease. Supplementary vitamin C is also required by active brood-stock fish in order not only to condition them, but also to increase the quality of gametes and the hatching rate of the fertilised ova.

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MANAGEMENT OF DIFFERENTIAL GROWTH AND CANNIBALISM

Krishna Sukumaran and A.R.Thirunavukkarasu

Introduction

In the larviculture of predatory fish species, differential growth and size variation are the common problems affecting drastically on the hatchery production. Good aquaculture practices aim at minimizing growth dispersion in order to achieve better growth and survival. Asian seabass (*Lates calcarifer*) fry reared under controlled condition face competition among individuals for feed and space resulting in uneven growth causing cannibalism (Mackinnon 1985). Cannibalism is both a cause and effect of this differential growth. Larger cospecifics will feed on the smaller ones leading to significant losses. It has been found that small size seabass fry become stressed in presence of larger cannibalistic conspecifics resulting in reduced growth rates and increased susceptibility to diseases. Size variations and cannibalism are closely linked and controlling the variations in size is one of the most effective ways to control cannibalism.

Some of the major causes of size variation are high population densities, feeding practices and initial size distribution which are essentially governed by genetic factors. The advantages conferred by virtue of its larger size results greater cannibalism by the individual. Genetic programming for larger egg size and early hatching results in early start of feeding resulting in a relative growth advantage. In case of European seabass (*Diacentrachus labrax*) the coefficient of variation wrt. weight was found to range from 10.2% (Person-Le Ruyet et *al.* 1993) and 15-20% (Kestemont *et al.* 2000). Size variation is the underlying cause for stable dominance hierarchies. These hierarchies are maintained by intra-specific agonistic interactions by the fish. Under conditions of larviculture it is important that such hierarchies are broken down.

Cannibalism or intra-specific predation is a common phenomenon among predatory fish. There are very few species of predatory fish where instances of cannibalism have not been recorded. Some of the species of fish where cannibalism has been recoded include *Clarias gariepinus, Centropomus undecimalis, seabass, Onchohynchus mykiss, Stizostedion vitreum, Oreochromis mossambicus, Moone saxatilis, Dicentachus labax, Cynoscion nebulosus, Scomber japonicus, Gadus mohus, Anguilla anguilla,Cyprinus carpio, Esox lucius, Epinephelus salmoides, Seriola quinqueadiata, Coryphaena hippurus.* In nature, cannibalism under some circumstances acts as a population regulation mechanism (in very low or highly productive environments) and can also act as a group selection process. Cannibalism is also believed to lead to increased robustness and vigor for the individual;it leads to a decrease in developmental time, increased somatic growth rate, enhanced fecundity and gonadal development. These benefits maybe good at the individual level but can have counter effects in the long run, especially from the viewpoint of larviculture. Cannibalism under culture conditions has been found to range from 15-90% successful cannibalistic attacks among fish of similar size lead to a drastic increment in growth of the attacker thus promoting further cannibalism and as a

consequence the cannibal never learns to take up artificial feed and thus leading to significant mortality in the long run.

There are two types of cannibalism tail first type (Type I) and head first type (Type II). Type I cannibalism usually precedes type II cannibalism because the larval mouth size has to increase so as to consume the prey head first. Type I cannibalism is not limited by gape width. Type I cannibalism is prevalent when size heterogeneity is low and the cannibals do not encounter prey small enough to be ingested whole unlike in the case of type II cannibalism where small prey are available and can be ingested whole due to the higher size heterogeneity. The predator can be 1.5 to 1.8 times the length of the prey but this varies with the species. In case of European sea bass the predator-prey size ratio has been recorded as 1.9. As a general rule it is believed that when the predator size is over 30%of the prey size cannibalism is facilitated. The overall impact of cannibalism on larviculture is mainly a function of initial size heterogeneity and also the timing of first emergence of cannibalism and proportion of deformed larvae. Study of size heterogeneity of the fish population to understand cannibalism can also be a little deceptive. For eg. high size heterogeneity among the individuals may be interpreted as a stage of intense cannibalism whereas actually it may only indicate a high cannibalistic potential from then onwards. Similarly low size heterogeneity can be wrongly interpreted as a population with low cannibalism whereas in actual it may be a group where the cannibals may have preyed majority of the smaller conspecifics creating a transient low size heterogeneity situation before cannibalism can be excreted again. However, as a thumb rule, high growth rates associated with high size heterogeneity indicates intense cannibalism whereas normal growth rates under conditions of low size heterogeneity reflects low cannibalistic pressure.

The chief influences on cannibalism can be classified as genetic, behavioral and environmental factors. Genetic factors are responsible for variations in growth rates leading to size heterogeneity, one of the key factors influencing cannibalism. Behavioral pattern is closely associated with the genetic and environmental factors. Social dominance is an important kind of behavioral pattern which leads to size variation, social hierarchy and aggressive responses. Some environmental factors when become limiting, affect the behavior and influence the rate of cannibalism. These include availability of food, availability of alternate prey, nutritional composition of food, population density, refuges, and water clarity, light intensity and feeding frequency.

Factors responsible for differential growth and cannibalism:

Food availability

It has been well established that food availability both spatially and temporally, is inversely related to size heterogeneity and cannibalism. It is interesting to note that in African catfish that as food availability declined territorial instinct increased and with a further reduction in food availability the fish took to cannibalism. In another study on the same species dealing with starvation and its impacts on cannibalism, it was found that the in starved fish there was an initial increase in cannibalism but with time it deceased has been attributed to a weakening effect in fish. However, abundant food availability can only help to reduce cannibalism but not eliminate it.

Food distribution should be uniform throughout the area of rearing environment; localized food distribution restricts access to food to smaller individuals which are intimidated by larger fish, thus preventing them from feeding. Nutritional quality of the feed and nutrient deficiencies can have a significant effect on cannibalism.

Initial size heterogeneity

This becomes a major problem in species where broods hatch over a longer interval of time. The earlier the hatching time, higher the survival and faster the growth, giving better competitive abilities to the young which ultimately grow larger and consume the smaller siblings. This phenomenon has been observed in case of European seabass (Kestemont *et al.* 2003). Hence, it is wise to separate early and late hatching larvae to minimize size heterogeneity.

Life stage

During the culture period, losses are more significant during early larval and juvenile stages when the fish have higher growth capacity than adults. The early life stages consume large rations relative to the body size, are prone to dispensatory growth which facilitates cannibalism.

Stocking density

Stocking density is seen to be positively correlated to cannibalism in many species owing to the increased probability of prey encounter. This has also been established in case of European seabass by Katavic *et al.* (1989) and Hatziathanasiou *et al.* (2002). However, like the effect of food availability, initially stocking density increase also results in increase in territoriality and agonistic behavior. But with further increase in stocking densities social dominance, aggressiveness and territorial defense seem to decrease leading to higher survival rate, but low growth rates. At low stocking densities the motivation to maintain territory often overweighs the motivation to forage explaining the low growth rates. Hence, during larviculture, it is important to determine optimum stocking densities.

Live food vs. dry food

Due to high costs involved rearing the larvae on live food, there are efforts to wean the fish as early as possible. It is found in a few studies that when fed with live food which is often the preferred food for larvae cannibalistic and territorial behavior were suppressed. When larvae are weaned to artificial diets, those larvae which wean early get a comparative growth advantage and this may also initiate cannibalistic tendency in some of the larger larvae.

Temperature

The effect of water temperature on cannibalism is poorly documented in culture environments. It is believed that the risk of cannibalism increases with temperature as there is a simultaneous increase in food requirement of the animal. Low

temperatures are associated with reduced appetite and low growth rates; hence the impact of cannibalism is expected to be reduced.

Light

In case of visual predators, the effect of cannibalism is subdued under low light conditions. So, rearing under low light conditions may be a way of managing cannibalism in visually oriented predatory species. Post larvae of European seabass were found to perform similarly under different light intensities but longer day lengths were found to be more suitable for larval rearing (Kestemont *et al.* 2003).

Turbidity

Turbid conditions simulate low light conditions and availability of refuges. These conditions are hence believed to reduce territoriality and cannibalism in the fish but this also leads to reduced browsing and feeding activity which ultimately hampers the growth of larvae.

Refuges

In a study on African cat fish, refuges were shown to significantly reduce cannibalism for both larvae and early juveniles. The impact of refuges on cannibalism needs to be studied for other aquaculture species.

Genetic control

There is evidence to believe that cannibalism is genetically controlled, however the subject is debatable. In this regard there are two hypotheses, natural-born killer hypothesis (some individuals are born as cannibals) and lottery winner hypothesis (some individuals get an edge after the initial competition and turn cannibalistic). Cannibalistic strains may arise in the process of domestication because the characters favored during selection by aquaculturists also seem to favor cannibalism eg. Fast growth may promote cannibalism via increased size heterogeneity. Cannibalistic tendencies may be more in one sex of a species which exhibits sexual dimorphism.

Mitigating differential growth and cannibalism in culture systems

The complete elimination of cannibalism in aquaculture especially larviculture is probably impossible, however adopting some of the following measures will help mitigate cannibalism.

- Optimal stocking densities should be determined at which cannibalism is reduced and sufficient economic productivity maintained.
- Resorting to satiation feeding can lead to reduction in size heterogeneity and cannibalism in culture systems

- It is important to understand the optimal feeding frequency for each species. An understanding of the time of the day for optimal feed acceptance will be beneficial in deciding these feeding frequencies. Feeding frequency may be increased from 3 to 6 times a day to reduce cannibalism.

- Uniform distribution of food throughout the rearing surface of the container will give equal opportunity for all individuals in the system to access food.

- Good quality artificial feed of optimum and uniform size helps to avoid nutrient deficiencies and avoiding cannibalism. Adequate amount of live food supplements may be provided as per need.

- Understanding the photic preference of the species and exploiting the light regime can be useful in reducing cannibalism.

- Regular grading of fish by size should be a regular management measure to remove cannibals and dominant individual. .

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NURSERY REARING

A.R.Thirunavukkarasu and M. Kailasam

1. Introduction

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. Nursery rearing is an important phase in the culture since this transitional phase can be used for acclimatisation and weaning to artificial feed and environmental conditions that will be available in the growout 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 rearing of sea bass needs special care because of the cannibalistic tendency and the differential growth of the fish, which are serious problems. Cannabolism is more common when the fish are around of 1 – 2 cm length in the first two months. Nursery rearing can be done either in the hatcheries or in the farm site.

2. Nursery Rearing in Hatcheries

Seabass fry of 25 – 30 days old in the size of 1.0 – 1.5 cm can be stocked in the nursery tanks of 5 – 10 tonn capacity circular or rectangular (RCC or FRP) tanks. Outdoor tanks are preferable. The tanks should have water inlet and outlet provision. Flow through provision is desirable. *In situ* biological filter outside the rearing tanks would help in the maintenance of water quality. The water level in the rearing tanks should be 70 – 80 cms. Good aeration facility should be provided in the nursery tanks. Nursery tanks are prepared a week before stocking. After filling with water 30 – 40 cm and fertilized with ammonium sulphate, urea and superphosphate @0, 5 and 5 gm (10 : 1 : 1 ratio) per 10 tonne of water respectively. The natural algal growth would appear within 2-4 days. In these tanks freshly hatched *Artemia nauplli* @00 – 1000 l are stocked after levelling the water to 70 – 80 cm. The nauplii stocked are allowed to grow into biomass (Refer Biomass production of Artemia) feeding with rice bran. When sufficient Artemia biomass is seen, seabass fry are stocked @00 – 1000 nos/m^3. The pre-adult *Artemia* would form good food for seabass fry. The fry would not suffer for want of food in the transitional nursery phase in the tank since the larvae are habituated to feed on Artemia in the larval rearing phase. Along with 'Artemia biomass' available as feed inside the tank supplementary feed mainly minced fish/shrimp meat is passec through a nesh net to make each particle of size of around 3 – 5 mm and cladocerans like *Moina* sp can also be given. The fish/shrimp meat feeding

has to be done daily 3 – 4 times. Feeding rate is 100% of the body weight in the first week of rearing. This is gradually reduced to 80%, 60%, 40% and 20% during 2nd, 3rd, 4th and 5th week respectively. Regular water change to an extent of 70% is to be done daily. The left over feed and the metabolites have to be removed daily and aeration should be provided. In a rearing period of 4-5 weeks in the nursery rearing, the seed will be in the size of 1.5 to 3.0 g/ 4-6 cm with survival rate of 60-70%. Adopting this technique at a stocking density @ 1000 nos/m^3 in the hatchery, survival rate upto 80% has been achieved. For better survival, regular 'Grading' should be done. Vessels/trough placed with different mesh sized nets can be used for grading. When the seed are left into the containers the seeds will be sieved in different grades according to the mesh size and seed size. Care should be taken that the fry are not injured while handling. If the number is less it could be manually done.

Major problem encountered in the nursery in the hatcheries is the pathological infection. Normally the infected fishes will be with inflammated opercular region, directly exposing the gills. With a whirling movement the fishes die within few hours. This may be due to bacterial or viral diseases. This problem is chronic during October – November months when the water temperature is less than 25^{0}C. As a precautionary measure, treatment with 2 ppm furozolidon can be done. To avoid such problems, the hatchery should be hygienically maintained. Water temperature of 28 – 29^{0}C is desirable.

3. Nursery Rearing in Growout Site

Rearing fry to stockable size seed in the hatchery itself have some problems. All hatcheries maynot have such facilities since the requirement of space will be 5 – 6 times more than larval rearing space. Maintenance requires additional man power, energy etc. Above all, transportation of large sized seed to culture site would be expensive. To avoid these problems nursery rearing in growout site itself can be done wherever possible.

3.1 Nursery Rearing in Ponds

Nursery ponds can be around 200-500 m^2 area with provision to retain atleast 70 – 80 cm water level. Adequate provision for water inlet and water drainage should be provided. Towards drainage side there should be slope. Suitable sized (normally 1 mm) mesh screen nets should be provided in the inlet side and outlet side to avoid entry of unwanted fishes and escape of the stocked fish respectively.

The pond is prepared before stocking. If there are any predator/pest fishes they have to be removed. Repeated netting, draining and drying the pond are done. In

case where complete draining is not possible, water level is reduced to the extent possible and treated with Derris root powder @20 kg/ha added or mahua oil cake @ 200-300 kg to eradicate unwanted fishes. Use of other inorganic chemicals or pesticides is avoided because these may have residual effect. After checking the pond bottom quality water is filled. If the pond bottom is acidic, neutralization is done with lime application.

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

Seabass fry is stocked @20-30 Nos/m^2. Stocking should be done in the early hours of the day. Fry should be acclimatized to the pond condition. Acclimatization for the pond condition is done as follows:

The fry in the transport container are emptied into another tank and the pond water is gradually added into the container. This process is continued for a day or two depending upon the difference in the parameters. When the water temperature and salinity in the pond and tank water reach same, fry can be released into the pond. Water is changed @30% daily. Supplementary feeding is done with chopped, cooked fish/shrimp meat. The larvae can be weaned to artificial feed at this stage. The feeding rate can be as mentioned in earlier. Excessive feeding should be avoided since excess feeding would deteriorate the pond condition and also promote filamentous algal growth. The excessive algal growth would deplete dissolved oxygen level in the early hours of the day leading to fish mortality. Hence, excessive algae if any should be removed.

3.2 Nursery Rearing in Cages/Hapas

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

Floating net cages/hapas can be in the size of 2 x 1x 1 to 2x 2x 1 m depending upon necessity. Cages are made with nylon/polyethylene webbing with mesh size of 1 mm. Fry can be stocked @00 – 500/m ^{2}. Feeding rate can be as that described to tank nursery. The net cages have to be checked daily for damages those may be caused by other animals like crabs. The net cages will be clogged by the adherence of suspended and detritus materials and siltation or due to foulers resulting in the restriction of water flow. This would create confinement in the cages and unhealthy conditions. To avoid this, cages/hapas should be cleaned everyday. Regular grading should be done to avoid cannibalism and increase the survival rate. Even in higher stocking density @00/m ^{2} farmer could get survival of 80% in the farm site when the fry were reared in hapas adopting the trash fish feeding and other management strategies mentioned above.

4. Conclusion

In the nursery rearing of seabass fry, since they fish exhibits differential growth and tendency of large fish feeding on the small fry (cannibolism) the most important component is grading and maintenance of uniform sized fish.

SEABASS HATCHERY MANAGEMENT PROTOCOL

R. Subburaj, G. Thiagarajan, A.R.T. Arasu, M. Kailasam and J.K. Sundaray

INTRODUCTION

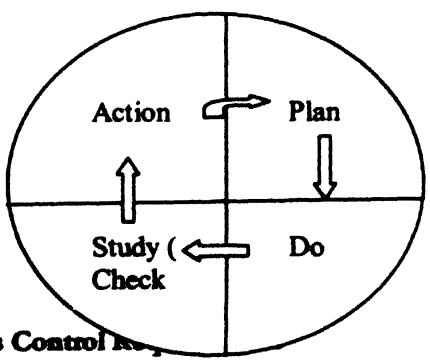
The quality seed production under controlled conditions depends upon the manner in which the hatchery is managed. A well maintained and managed hatchery is expected to deliver quality seed. Apart from protocols under controlled breeding like development of viable broodstock, maturation facilities, incubation facilities, larval rearing, live feed culture and nursery rearing facilities, the hatchery has to be maintained and managed with utmost care. . Following the systematic data sheet and protocol sheet for smooth and hustle free operation of each units of hatchery be used effectively for quality seed production. A model sheets for each unit of hatchery is briefly explained.

General Management in Hatchery

Hatchery facilities shall be operated to maximize the quality seed production, the hygienic protocols should be strictly followed in each and every stage of seed production, right from the brooder selection, spawning, incubation, stocking, larval rearing and live feed culture. The supporting system of hatchery like pumps, filters, aerators, air and water net work should be monitored and maintained regularly for successful operation of the hatchery.

Hatchery Management Responsibilities

Hatchery management involves strategic planning, execution and involvement to achieve the targets. The hatchery management responsibility is briefly explained by a wheel chart developed by Li deming. The PDSA Cycle (or the Deming cycle, Deming PDSA approach is that improvement in quality results from continuous, incremental turns of the wheel (Brophy and Coulling 1996).



Breeding Process Control

- Broodstock Procurment and Qarantine

Procure brooders from all available sources like wild collection or farm reared and do proper quarantine before stocking as broodstock.

- Activity Diagram

Plan breeding programme schedule according to the hatchery capacity, brooder availability, no. of seed targeted and breeding seasons.

- Broodstock rearing procedures

Create data sheet for broodstock procurement, broodstock management such as water quality, feeding and health management

- Sanitary Management

Ensure healthy environment for broodstock rearing following standard sanitary protocols like water exchange, bottom cleaning, removal of unfed feed and periodical monitoring of fish for parasites and pathogens.

- Genetic Selection of fish

Hatcheries should have a reliable and sufficient supply of good quality fish eggs. Hence, hatcheries have to establish their own broodstock units with different age groups and from different sources like wild, farm reared and hatchery reared are kept under long term stocking conditions.

WORKING SHEET FOR BROODSTOCK MANAGEMENT TANK NO:

No. of Fishes : Male **nos. Wt.** **Female nos. Wt.**

Date of Transfer **Age**

Water Quality		
Temperature		
Salinity		
D.O.		
pH		
Ammonia		
Water exchange		
Tank management		
Water exchange		
Bottom cleaning		
Feed management(Feed type, qty consumed)		

Larval Rearing protocols and Work plan sheets

Create data sheets for the following larval rearing procedures

- Weaning protocols
- Nursery protocols
- Water management
- Maintenance procedures
- Biofilter management
- Bacterial quality management
- Feed bacterial quality
- Night feeding

LARVAL REARING SECTION. DAILY WORK PLAN

Tick mark the relevant cell when the work is completed

Work Parameters	Time (hrs)																	
	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23-06
Algae distribution																		
Rotifer distribution																		
Artemia Nauplii distr.																		
Artemia meta dist.																		
Artemia feed distri.																		
Rotifer Check																		
Bottom cleaning																		
Bottom Purge																		
Skimmer cleaning																		
Oultlet screen ch.																		
Temp. Salinity, pH Reading																		
D.O. ppm																		
NH_4																		

In Larval section check the following frequently :

Outlet screen : For clogging, mesh size, disturbtion to larvae

Skimmer : Air flow speed, collection of dust, clean inner surface, no dead larvae logging

Water Surface : Check for greasy or rotifer layers

Water flow : Check inlet and outlet water flow

LIVE FEED CULTURE PROCEDURES AND WORKPLAN SHEETS :

- Qality care
- Maintenance of sheet for rotifer rearing
- Sheet for sample stocking

- Sheet for sample management
- Define the sanitary management

Daily work plan and procedures for Algal section

Production of microalgae for the greenwater in larval rearing tanks and to inoculate one new rotifer tank daily. To ensure continuous supply of algae for continuous culture and maintenance of rotifers, it must be inoculated daily to keep the production chain:

Daily Work Plan for Algal section

Time (hrs)	Culture room controls	Culture work	Upscaling	Harvesting	Remarks
08.00	Gen. overview Temp. A/C	Media requirement .	Gen.overview	System control	
09.00		Glass vess.	Glass to bottle	Gv.	
10.00				tank	
11.00		preparation	Bottle to tank		
12.00	U.V. filter			cleaning	
14.00		inoculum			
16.00		Next day requ.		Filling/inocul.	
17.00	Gen. over view				

Micro Algae culture file

Date	Age (days)	Vol (lit)	Density (cells/ml)	Light (lux)	Air	pH	Tem °C	Salinity Ppt	Contamin.	Use	Rema

Rotifer Section
Collection of Rotifer for feeding

Tank no.----- to ------ larval tank
Tank no.----- to ------ larval tank

For Enrichment of rotifers for fish larvae :

filter _litres of tank _
Stock _litres of tank _

Inoculation from tanks:

filter _litres of tank _to inoculate tank _
filter _litres of tank _to inoculate tank _

Inoculation from stock (SS):

filter _litres of SS _to inoculate tank _
filter _litres of SS _to inoculate tank _

Addition of algae:

add _litres of SS _to tank_
add _litres of SS _to tank_

Filling 30 ppt treated water to tanks::

add _litres of seawater to tank _
add _litres of tap water to tank _
add _litres of seawater to tank _
add _litres of tap water to tank _

Settling and purge of tanks

tank _
tank _

Discharge:

tank _
tank _

ARTEMIA SECTION

Product Inspection and Testing

Record the receipt of artemia cyst, artemia production and supply of nauplii to larval rearing section.

Data sheet for Artemia Incubation and Hatching

INCUBATION (Cyst)					HATCHING (nauplii)					
Date	Time	Tank	Litres	Cyst (g)	Date	Time	Tank	No./ml	Million	Hatching

Enrichment			Harvest		
Tank	Selco		No./ml	Million	Survival%
	Time	Dose			

Inspection of hatchery Equipments

The infrastructure and allied facilities in hatchery should be regularly monitored, daily maintenance and follow up action of every stage should followed for regular operation of hatchery during production and shut down periods.

The following points are to be followed in hatchery :

- Listing all equipments and places
- Calibration planner
- Calibration procedures
- Stores inspection schedule
- Inspection and test status of products
- Control of non conforming products
- Handling, Storage and Delivery
- Control of quality records
- Internal quality audit requirements
- Training requirements
- Service Requirements
- Statistical techniques

Maintenance of Hatchery Infrastructures Chart

Technical Maintenance Plan

Inventories	Period											
	July	Aug	Sep.	Oct.	Nov.	Dec.	Jan	Feb	Mar	Apr	May	June
Sea water inlet												
Pumping Station : Pump 1 : Pump 2 : Pump 3 :												
Inlet pipe Pipe 1 Pipe 2												
Principal Valves												
Hatchery pipe												
Brood- stock pipe												
Growing pipe												
Inlet water filters												

U.V. Lamps												
Seawater & Freshwater pipe B.S. sect. Incubation Phytoplankton Zooplankton Larval Nursery												
Filters and U.V. lamp in all sections												
Close circuit pump in B.S, Larval and Nursery												
O_2 Network												
Air supply net work												
Electrical net work												
Heaters												
Coolers/ A.C												
Electric Generators												
Alarms												
Vehicles												

Each work should be marked against relevant months

Protocols in Hatchery Maintenance

1. **Responsibilities :** Assure the technical maintenance of hatchery

2. **Organisation of technical works**

- Definition of technical work for the hatchery in consultation with hatchery manager
- Order the missing spare parts
- Imagine and conceive new techniques

3. Spare Parts Management

- Assure the spare parts availability

- Stock control check for fuel, gas and feeds
- Efficiency for technical work organization

4. Daily Work Protocols

1. Analyse night data and eventuality
2. Observation of Larvae
3. Clean live food tanks
4. Clean oil skimmers
5. Give food as soon as possible
6. Tank data – Physio-chemical parameters
7. Siphon the bottom
8. Make purge – bottom line clean
9. Drive the tank protocol
10. Quality control
11. Hygiene management – Follow larval hygiene plan
12. Prepare the data for night

NUTRITION AND FEEDING OF ASIAN SEABASS IN HATCHERY, NURSERY AND GROW-OUT PONDS

S. Ahamad Ali, J. Syama Dayal and K.Ambasankar

INTRODUCTION

Asian sea bass (*Lates calcarifer*) has emerged as an important candidate finfish species for aquaculture in the Brackishwater sector. Availability of seed and appropriate feed are two important prerequisites for the development and propagation of aquaculture of any fish species. After considerable efforts and extensive research the Central Institute of Brackishwater Aquaculture succeeded in developing captive brood stock and seed production technology for Asian seabass. Research efforts on nutritional requirements and development of suitable formulated feeds have been in progress simultaneously at CIBA.

NUTRITIONAL REQUIREMENTS

Investigations on Asian sea bass **(Barramundi)** (also known as **Bhetki** in Bengal) have been mainly concentrated on energy nutrient requirement in the diet. Recently information on micro-nutrient needs such as vitamins has started coming in.

Protein

Seabass being highly carnivorous fish showed a dietary requirement of 45 – 55% protein as determined by different workers (Cuzon and Fuchs, 1988; Tucker et al, 1988; Wong and Chou, 1989). Subsequently Catacutan and Coloso (1995) suggested 42.5% in the diet of the fish. Experiments conducted in CIBA with different level protein feeds on the young-ones of seabass showed a protein requirement of 43 % for this fish. The protein quality in the feed influences the requirement. Most of the finfish show the requirement of the same ten amino acids (arginine, histidine, isoleucine, leucine, lysine, methionin, phenylalanine, threonine tryptophan, tyrosine or valine) as essential. However, determination of quantitative essential amino acid requirement would help in assessing the protein requirement more accurately.

Lipid

The quantitative lipid requirement in the diet of seabass is estimated to be in the range of 6-18% (Cuzon and Fuchs, 1988; Tucker et al, 1988; Wong and Chou, 1989). Catacutan and Coloso (1995) suggested 10% lipid (in combination of 42.5% protein) in the diet for the juveniles *L. calcarifer* for good growth and FCR. When the lipid level was raised to 15% in the diet the protein sparing effect was not observed in this fish. The highly unsaturated fatty acids (HUFA) of n-3 series are reported as essential for sea bass

(Buranapanidgit et al., 1988) suggesting its marine species characteristics. The requirement for the n-3 fatty acids in the diet is suggested to be 1.72% for this fish. Deficiency of HUFA in the diet caused red colouration of the fins in the fish fed such diets. Phospo lipid in diet is found to improve the survival of seabass larvae. It can be used up to 8% in the larval diet

Energy

Carbohydrate levels of 10 –16 % were suggested in the diet of seabass (Cuzon and Fuchs, 1988; Tucker et al, 1988; Wong and Chou, 1989). Subsequently Catacutan and Coloso (1995) suggested 42.5% and 10% lipid with a protein – energy ratio of 128mg protein/kcal as optimum for the juveniles *L. calcarifer* for growth and good FCR and PER. Experimenting with carbohydrate and lipid for juveniles of Asian seabass, Catacutan and Coloso (1997) reported that best growth and FCR were observed in fish fed with 20% carbohydrate (bread flour as source) and 12% or 18% lipid (cod liver oil and soybean oil in 1:1 ratio).

Summary of energy nutrient requirements for seabass :

Nutrient	Requirement in diet
Protein	45 – 55%
Lipid	6 - 18%
Fatty acids	
(n-3 HUFA essential)	1.72%
Carbohydrate	10 – 20%
Protein : Energy ratio	128mg protein/kcal

Thus the information on the dietary nutrition of seabass started coming. Vitamin C in the diet is found to be beneficial for improving growth and FCR in Seabass. About 1.0 to 1.5% of Vitamin C is required in the diet of this fish. The dietary requirements of other individual vitamins and minerals are still not known.

FEEDS AND FEEDING OF SEABASS

Feeds and feeding of larvae in hatchery and nursery

Larvae of finfish and shellfish are generally fed with live food organisms (Phyto or zoplanktons or both) in the initial phase; Investigations revealed that the developing larvae do not have the full complement of digestive system developed. The larvae of seabass are no exception to this. Studies conducted at CIBA on the metabolic changes and nutrient turn-over in developing Seabass larvae revealed that the growing larvae require the essential aminoacids leucine and lysine at higher levels in the larval diets

(Syama Dayal et al., 2003). Being carnivorous, seabass larvae are fed with zooplankton such as rotifers for the first two weeks post hatch (PH) and then switched over to brine shrimp (*Artemia*) nauplii. The size of the rotifers plays an important role in successful rearing of the larvae. Super small size rotifers are preferred for feeding seabass larvae. Since Artemia is an expensive live-food, its replacement by prepared diets has assumed significance in the hatchery and nursery rearing of fish larvae. In this context formulated micro particulate and microencapsulated diets have been successfully used for feeding the growing fish larvae.

In the case of seabass it is all the more important to wean the larvae to prepared diet so as to continue them in grow-out system where formulated feed has to be used for their culture. Seabass is very conservative in its feeding habit. The larvae of seabass are successfully weaned to prepared diet in CIBA by adopting co-feeding technique. The larvae after 18 to 19 days PH are first introduced to prepared diet in semi-moist form by co-feeding with boiled fish meat. The fish meat is gradually replaced with prepared diet and in about seven days the larvae are totally on prepared diet (Ahamad Ali et al., 2000). The larval weaning diet is prepared using marine fish and soybean meal containing 45-50%of crude protein. This diet is successfully used for rearing the larvae in hatchery and nursery. Further improvements have been made in the larval diets by incorporating specific amino acids and phospholipids. Frequent feeding of larvae in hatchery and nursery is essential for achieving good survival rates. The larvae, trained on prepared diet are given to farmers for further grow-out culture. Dry particulate feeds in the size range of 200 to 300 micron size containing 50-55% protein are being evolved at CIBA for the larvae of seabass. Such feeds are used in Australia, Thailand, Taiwan and other East Asian countries.

Feeds and feeding of seabass in grow-out culture

In some of the East Asian countries and also in India seabass is cultured in grow-out ponds using low value fish (trash fish) and tilapias in fresh condition. Since procurement and storage of these feed-fish is not only laborious but also quite expensive. Hence formulated feeds are essential for the propagation of large-scale farming of seabass.

Asian sea bass is cultured in Australia and Thailand using formulated feeds (Mackinnon, 1989;Boonyaratpalin, 1991). As in the case of other carnivorous species, feed formulations for seabass utilize marine fish resources (for meeting protein requirement) and fish oils along with plant protein sources. The animal ingredients are kept above 60%of the formulation to get protein levels in the range of 45-52% Experiments conducted at Muttukadu field laboratory of CIBA had shown that feeds with substantial fishmeal component (30-40%only have good acceptability for seabass. Higher the proportions of fishmeal better the acceptability. The texture and size of the feed effects acceptability of the feed. If the flavour and texture of the feed are not to

the liking of the fish, it spits out such feed even after taking it into its mouth. For keeping higher protein levels in the feed, use of animal protein sources such as fishmeal is inevitable. However, plant ingredients such soybean meal and other oil seed residues may be utilized in the feed formulations. For providing the polyunsaturated fatty acids (PUFA) use of marine fish oils should be included in the feed formulations. Studies conducted at CIBA on the feed attractants for seabass revealed that the amino acid - glutamic acid and trimethyl amine are useful as feed attractants for this fish (Syama Dayal et al., 2002).

Seabass feeds on moving prey; hence the physical design of the feed plays a very important role. The fish readily accepts soft semi-moist feeds with appropriate size to swallow vis-à-vis the size of the fish. The lower lip of the fish is curved slightly upward, which is disadvantageous for biting the feed. Floating and slow sinking pellet feeds are more suited for feeding seabass. Such feeds are generally processed in extruders.

Extruder technology

The basic components in an extruder are a barrel fitted with a die plate and a screw shaft conveyer, which is connected to a high-speed motor. The feed mixture is fed into an extruder by proper arrangement of water/steam injection facility. The extruder operates at high pressure (14-98 kg/cm^2) and steam (Pressure 5 - 7 kg/cm^2) injection. Depending upon the characteristics of the feed mixture and moisture content, the pressure develops before the material passes through the die. Because of this the temperature rises and the material is forced through the die and the pressure suddenly drops. The temperature of the material rises to 110 - 130°C for a short spell of time and cooks the food, gelatinizing the starch present in the feed mixture. This imparts good binding and water stability to the resultant pellets. However, the pellets expand as they come out of the die due to sudden drop of pressure and air gaps develop inside the pellet, which makes them float or sink very slowly. This is an excellent process for producing floating pellets for finfish culture. By adjusting the pressure in the barrel and moisture in the feed, it is possible to prepare sinking pellets by extruder. The new generation extruders are made with twin screw-barrel arrangement, which are more versatile for feed manufacture. The size of the pellet diameter ranges from 0.5 mm to 8.0 mm.

The characteristics of extruder pellets are

1. Reduction in pellet disintegration and loss in water.
2. Increases starch digestibility due to good cooking
3. Can be worked with higher moisture and oil (fish oil) levels in the feed.

4 Extruder pellets float or sink slowly.

5. Making charges for extruder pellets are higher due to high cost of extruders

At CIBA, formulated feeds developed as floating and sinking pellets were successfully tested in grow-out ponds and grown the fish to 500 g in six months.

The fish should be fed at the rate of 10% of their body weight to start with. After four to six weeks the feeding rate may be reduced to 8%. As the fish grow in size the feeding rate should be gradually reduced to 5%, 3% and even 2% finally. The total biomass in the pond should be periodically estimated by a suitable means (by caste netting) for adjusting the feed. The entire quantity of feed in a day should not be given at one time. It should be divided and fed 3-4 times a day.

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CULTURE OF ASIAN SEABASS (*Lates calcarifer*)

G. Biswas & J.K.Sundaray

1. Introduction

Asian seabass *Lates calcarifer*, commonly called as 'Bhetki' is a large sized Sea Perch, native to the Indo-Pacific region and its range extends north as far as Taiwan, south to the eastern Australian coast, east to Papua New Guinea and as far west as the Persian Gulf (Greenwood, 1976 and Tucker *et al.*, 2002). Seabass is both caught commercially from the brackishwater estuaries, backwaters and nearshore waters by artisanal fishermen. This is an excellent brackishwater table fish, highly relished due to its flesh quality. The euryhaline nature of this species enables its culture in fresh, brackish and seawater environments. Additionally, seabass has the uncommon ability to synthesize long chain omega-3 fatty acids, whose contribution to human health has been found to be increasingly important. Commercial seabass culture in both ponds and cages has been carriedout profitably in Southeast Asian countries since the late 1960s and in Australia since the early 1980s (Corey Peet, 2006). In India, the culture is mostly dependent on natural seeds. A breakthrough in seabass breeding and seed production for the first time was achieved by CIBA, Chennai during the late 90s and its year-round seed production technology has already been developed, but commercialization is yet to be undertaken by entrepreneurs (Thirunavukkarasu *et al.*, 2001). Seed production started at the Rajiv Gandhi Centre for Aquaculture (RGCA) of MPEDA in Tamil Nadu and Pancham Aquaculture in Thane, Maharashtra following the CIBA technology is a promising step forward to meet the much awaited seed demands of farmers (Vartak and Belsare, 2004). In brackishwater aquaculture sector, with the setbacks in shrimp farming caused by the dreaded white spot syndrome viral (WSSV) disease in India, the aquafarmers are gradually turning to finfish culture and in this context *L. calcarifer* is the most preferred species of choice. Similarly, in freshwater sector, as the market price for IMC and other carps has become almost static and the margin of return is reducing with the regular increase in production cost, the farmers are inclined towards culture of some high-value, high-demand fish like seabass.

2. Status of sebass production

Worldwide aquaculture production of seabass began in the late 1960s (FAO, 2004). Bhetki are currently farmed in Australia, Southeast Asia (Malaysia, Indonesia, Thailand, Brunei Darussalam, Singapore), Taiwan, Israel and more recently in the United States. In 2004, the world aquaculture industry produced 29,884 metric tons (mt) of seabass product, valued at U.S. $7,733,000, out of which 3,479 mt from

freshwater, 24,580 mt from brackishwater, and 1,825 mt from saltwater (FIGIS, 2006). Thailand was the largest producer (14,550 mt), followed by Taiwan (4,985 mt), Indonesia (4,663 mt), Malaysia (4,001 mt), Australia (1,567 mt), Singapore (77 mt), Brunei Darussalam (43 mt) and Israel (15 mt) (FIGIS, 2006). Vietnam is also producing seabass, however, their production is not registered in the FAO database. Until recently, China was a producer of *Lates calcarifer* (peak production of 224 mt in 1993) but no data is available for 2004 (FIGIS, 2006). The vast majority of worldwide production is consumed domestically in producing countries, with only minor quantities being exported (For e.g., in Australia: 97%domestic consumption, 3% export). In the Indian context, traditional culture of seabass in large impoundments known as "*bheries*"where the tidal water influence is felt along the Hooghly-Matlah estuarine systems has been carried out since 1829 (Lovatelli, 1990) and though, this type of farming is prevailing in east and south west coast, improved culture has started in some places very recently and no organized data are available on aquaculture production.

3. Seabass culture methods

Seabass is cultured in different ways, *viz.* traditional culture practice and improved culture methods. Improved culture techniques include pond culture (polyculture and monoculture), culture enclosure (cage farming). The improved culture techniques for seabass farming are of recent origin and gaining rapid popularity among farmers.

3.1 Traditional culture practice

Brackishwater tidal wetlands, namely mudflats, swamps, marshes, paddy fields etc., situated in low-lying areas are used traditionally for culturing fish and shrimps. These water areas are fed by tidal water from nearby creek/ canals/ rivers. The fish juveniles and shrimp seed entering through tidal water as per their seasonal availability were allowed to grow in polyculture mode without provision of any other inputs. The stock is left at the mercy of natural environment and the prey available and vulnerable for predation by the supplementary stocking of seabass and for feed stocking of tilapia is followed. But in most cases supplementary feeding is not practiced as the entire production system is dependent on utilization of natural productivity (Alagarswami, 1995). Harvesting is done after 6-8 months of culture and large sized seabass are found with less in numbers compared to improved culture systems. In this system production ranges from 500-1000 kg/ha/year.

3.2 Improved culture techniques

As seed is regarded as the main critical input, in the areas where wild seeds are available, the farmers go for improved farming system of seabass culture.

3.2.1 Polyculture system

Poyculture is a farming practice where two or more species of fishes are reared together. This method is a modification and improvement over the traditional method.

In the traditional method the quality and quantity of the prey (foods) fishes/crustaceans entered the pond is not known and the water quality is not monitored, whereas in this improved polyculutre method, the food required for seabass is produced in the pond itself and seabass seed is stocked thereafter. The pond for seabass polyculture is prepared first, following eradication of unwanted organisms and application of manures and fertilizers. The pond is at first dried, ploughed and limed @-200 kg/ha to maintain soil pH above 6.5. Then the pond is manured with raw cattle dung @10 ton/ ha based on the soil organic carbon content. Pond is filled to a depth of 60-70 cm and fertilized with urea @5-100 and super phosphate @5-100 kg/ ha according to soil available nitrogen and phosphorus levels, respectively. After 3-4 days of fertilization, once the water colour becomes light green, forage fishes are introduced. Tilapia, *Oreochromis mossambicus* and *O. niloticus* which are omnivore and prolific breeders are the best suited candidates as forages for polyculture with Bhetki as the primary crop. Tilapia fry are introduced first @,000-20,000/ha, 1-2 months prior to seabass seed stocking. Tilapia is fed with cheap feed mixture consisting of rice bran and mustard oil cake at 1:1 ratio. The tilapia grows and breeds gradually. As a result, in the same pond adult fishes, small fry, late fry, fingerlings, juveniles are found and served as food for seabass. In this pond, seabass seeds of 3-5g size are stocked @000-10,000/ha. To provide more food, at the time of water exchange, tidal water is directly pumped into the pond so that many small fishes and crustaceans enter the pond and serve as food for seabass. If there is reduction in forage fish noticed, it is supplemented with further introduction. This practice is more sustainable. The culture period lasts for 8-12 months. In this practice production up to 3 to 4 ton/ha is achieved.

Owing to its euryhaline nature, it is also cultured in freshwater polyculture systems with Indian major carps, medium sized carps, barbs and tilapias. Juveniles of IMC, *Puntius javanicus* and tilapia serve as desired food for seabass. After 8-10 month culture a total production of 1.5 to 2.5 ton/ha is obtained.

3.2.2 Monoculture

Monoculture of *L. calcarifer* in ponds is a well developed aquaculture industry in Taiwan (Boonyaratpalin and Williams, 2002). In India, it is practiced in some pockets where cheap trash fish as feed is available in plenty. Seabass seed of size 2g and above are stocked @10,000-15,000/ ha in well-prepared culture ponds (pond preparation similar to polyculture). In this system the stock is totally raised on supplementary feed. In wild, seabass prefers live food. So the fish is weaned to accept dead trash fish. Stocked fishes are fed with minced flesh of cheaper trash fishes collected from landing centres. Seabass does not feed at pond bottom, so the chopped trash fish is broadcasted slowly twice a day and the sinking feed material is engulfed actively by seabass. Feed is provided *ad libitum* at not more than 100% of total biomass initially and then gradually decreased to 10% at the last phase of culture. In this method, after a culture period of 8-10 months, seabass attain average size of 800g with a survival rate of about 60-70% and a production of 2.5 to 5 ton/ha is achieved. For the trash fish feeding, feed conversion ratio of 6-8 is obtained on wet weight basis.

3.2.3 Enclosure culture (cage farming)

Open net cage culture of Bhetki was first established in Southeast Asia in the late 1970s and continued to expand rapidly (Tendencia, 2002 and Alongi *et al.*, 2003). Cage culture technology is an eco-friendly culture method and can be adopted in open waters for which soil characteristics and basin topography do have any significant role. Bhetki fingerlings after grading and acclimatization are stocked in the grow-out cages. There are two types of cages, floating and stationary cages. Floating net cages are attached to wooden frames and kept afloat using plastic drums with anchors, whereas stationary cages are fixed enclosures which are fastened to wooden poles erected in the water body at the corners. In the cages, 10-15g fish are stocked @40-50 nos./m^3 initially. When the stocked fingerlings attain of about 150-200g, they are thinned to 15-20 nos./m^3 and transferred to different cages of required mesh size. Cage culture is normally done in two phases- till attaining of 150-200g in 2-3 months and thereafter upto 600-800 g in 5 months. Feeding in cages is done with either extruded pellets @ 2% or trash fish @5-5% at decreasing rate with the progress of culture period. Frequent size grading and culling of larger individuals are performed for obtaining high production. Here production level of 15-20 kg/m^3 can be obtained with normal management protocol. In India, cage culture of seabass has not yet gained the shape of commercial scale production. The reports whatever available are of experimental kind. Recently, RGCA has demonstrated a production of 12 ton/ha seabass from cage culture systems at their Aquaculture Demonstration Farm, Puducherry (MPEDA, 2007).

3.2.4 Recirculatory system

This type of seabass farming is undertaken outside the tropical areas like southern Australia, northeastern U. S., the Netherlands, Ireland etc. For closed recirculation, constant filtration is required to remove solids and particulate material from the water. The biological and mechanical filtrations act in concert to reduce water consumption down to 10-20%of standing culture volume per day. The treated wastewater from this culture system can be beneficially reused for irrigation and fertilization of agricultural crops (ANON, 1999).

4. Conclusion

Since 1970s seabass has been identified as a potential aquaculture species and farming activities have spread over many of the South-East Asian countries. In India, aquaculture activities are mainly concentrated on freshwater species namely Indian major carps and Chinese carps, shrimp and scampi. With the great export potential and domestic markets, India has not been able to make any significant contribution for seabass aquaculture. The farming is presently confined to a small-scale level in scattered way with potential to use vast resources. CIBA has already developed the seed production and culture technologies. Hence, the freshwater and brackishwater resources can easily be utilized for seabass farming by adopting these technologies.

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FARMING PRACTICES OF ASIAN SEABASS *LATES CALCARIFER*

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Asian seabass is one of the delicious table fishes. Amongst the cultivable fishes in India, seabass fetches higher price in domestic market varying between Rs.70-170 per kg depending upon the size, the availability and season. It is extensively cultured, in South East Asian Countries like Thailand, Malaysia, Singapore Australia. In India, culture of seabass in a limited scale is carried out in the traditional farms in coastal area. In the aquaculture operations compared to the carp culture in to freshwater or shrimp culture in brackishwater ponds, seabass culture is still a small proposition. The main reason for this is that the market for shrimp is global while the market for seabass is largely regional. However seabass is the only species, most commonly farmed amongst fin fishes in South East Asian countries because the seed can be produced in the hatchery economically throughout the year at reasonable price. Culture of seabass is relatively easy and dependable with fewer risks. Based on case studies, in Thailand it has been estimated that the production of seabass culture was 20.5kg/m^3. The price of seabass is US$1.27 per kg. The total income from the cage is US $46.49 per m^2. The rearing cost is US $24.15. The net return is US $22.34 per m^3. In the culture operation the fixed cost in cage culture is only 5.9%The variable costs such as feed, seed, labour etc cost 94.1%Feed alone cost 63%followed by seed cost. Seabass, the value added finfish can be considered as a complementary to shrimp for the sustainability of brackishwater aquaculture. It can also be considered as a species for freshwater aquaculture for enhancing the returns to the farmers since seabass fetches a higher price compared to that of carps. Seabass can be cultured in salt water and freshwater ponds and cages.

Traditional Culture

Seabass is cultured in the ponds traditionally as an extensive type culture throughout the areas in the Indo-pacific region where seabass is distributed. In low lying excavated ponds, whenever the seabass juveniles are available in the wild seed collection centers (For eg. April June in West Bengal, May-August in Andhrapradesh, Sept-Nov. in Tamil Nadu), May to July in Kerala and June-July in Maharastra) juveniles of assorted size seabass are collected and introduced into the traditional ponds which will be already with some species of fish, shrimps and prawns. Forage fishes like Tilapia will also be available in these type of ponds. These ponds will have the water source from adjoining brackishwater or freshwater canals, or from monsoon flood. The juvenile seabass introduced in the pond will prey upon the available fish or shrimp juveniles as much as available and grow. Since, seabass by nature is a species with differential growth are introduced into the pond at times of food scarce, the larger may resort to feed upon the smaller ones reducing the number.

Seabass are allowed to grow for 6-7 months of culture period till such time water level is available in these ponds. and then harvested. At the time of harvesting there will be large fish of 4 to 5 kgs as well as very small fishes. This is a common scenario in many coastal area. In this manner production upto 2 ton/ha/7-8 months

have been obtained depending upon the number and size of the fishes entered/introduced into the pond and the feed available in the pond.

However, this practice is highly unorganized and without any guarantee on production or return for the Aquaculturists. With the advances in the technology in the production of seed under captivity assuring the supply of uniform sized seed for stocking and quality feed for feeding, the seabass culture is done in South East Asian Countries and Australia in more organized manner.

The major problem in the development of seabass aquaculture in India is the availability of seed in adequate quantity and the time of need and quality feed for nursery rearing and growout culture. The former has been overcome and the technology package for the seed production of seabass under controlled conditions is available. The suitable feed for the culture of seabass is being developed. The seed production technology developed by CIBA has already been commercialized and the feed technology will be ready shortly. These technological improvements in the seabass culture have motivated the farmers to select seabass as a candidate species for aquaculture. Farmers have been adopting improved farming practices in seabass culture.

Improved Seabass Culture Methods

The traditional culture method is improved with stocking of uniform sized seed at specific density and fed with low cost trash fishes/formulated feed of required quantity. Water quality is maintained with exchange periodically.Fishes are allowed to grow to marketable size, harvested and marketed for high unit price. Seabass culture can be done in more organized manner as a small-scale/large scale aquaculture in brackishwater and freshwater ponds in cages.

Pond Culture of Seabass

Seabass is cultured in the ponds adopting poly culture method or feeding with low cost fishes like Tilapia/oil sardines or with extruded floating pellets.

Poly Culture Method

This is an improvement over the traditional method, where the feed, the live fishes, shrimps are deliberately allowed in to the seabass culture ponds to serve as facilitating feed for the seabass in the pond. In the traditional method there is no control over the quantity and quality of the feed entering the ponds which may or may not be adequate. At times of scarcity for feed, the seabass may resort to cannibalism resulting in low survival and production though few fishes will be large size. Under polyculture method the feed in the form of forage fishes are produced in the culture ponds itself and made available to the seabass fish to prey upon as and when it requires.

Pond preparation

The pond proposed for seabass seed stocking is dried, tilled and levelled, manured with raw cow dung @5,000 kg/ha. If required, lime is added @100-200kg/ha

to adjust the soil pH to above 7. Fertilizers like Urea @10kg/ha, Super phosphate @5kg/ha can also be added to enhance the algal bloom in the pond.

Water from the adjoining water sources seawater/freshwater is filled to a depth of 60-70cms in the pond. After sufficient algal bloom (light green in colour) is observed, in the pond forage fished are introduced.

Forage Fishes

Forage fishes, which can be used as natural feed, should be

a. Prolific breeding habit
b. Should not be a predator
c. May be a pest in the culture system
d. Should be lower in the food chain. Preferably herbivorous/omnivorous
e. May not be fast growing but can adopt to high stocking density
f. Should be palatable to the seabass

Fishes like *Seratherodon* (Tilapia) *mossambicus* qualities for a forage fish.

Forage fish/Feed fish stocking

Fishes like Tilapia can be introduced @20-25 thousand per ha. in different sized ranging from juveniles to pre adults into the pond. The average weight of the fish should not be more than 30 gms so that the biomass available at the time introduction will be around 600 kg/ha. Stocking of forage fishes should be done in the seabass culture pond a month ahead of seabass stocking. The water level in the pond may be maintained around 80-90 cm. Subsistence level of feeding with cheaper ingredients like Rice bran and ground nut oil cake @3% of the biomass can be done. Being a prolific breeder in habit, the forage fish *Tilapia* will start breeding in the pond and at given point of time in the proposed seabass culture pond, there will be different size groups like hatchlings, fry, fingerlings, juveniles and adults.

Stocking of Seabass

In the pond with natural forage feed, seabass seed of 2-3 gms can be stocked. Stocking density can be between 10,000 and 20,000/ha depending upon the management capacity of the farmers like water change and feeding at later stages. Seabass seed should be uniform in size to avoid differential growth in the culture pond.

Pond Management

The water level in the pond may be raised to at least 1 m depth. Water quality should be monitored regularly. About 40% water exchange may be done once in a week

to keep the water quality. In the pond, the feed for seabass will be the forage fishes. For the forages fishes, supplementary feeding may be continued.

The Seabass stocked will feed upon the hatchlings fry and fingerlings of the fry initially and later on the adult fishes also. Since, seabass has got higher growth potential than the forage fishes they will dominate in the pond. After 3-4 months there will not be any forage fish in the pond. At that stage, fresh stock of feed fishes should be introduced.

Production and Economics of Polyculture

Trial conducted on the culture of seabass adopting polyculture method yielded around 4.0 tons/ha for 11 months culture duration. Hatchery produced seabass fry of 1.0 cm were reared in hapa nursery at the culture pond site. After one months rearing the fry attained average size of 1gm. In a 1.0 ha area pond 8,000 seed of seabass were stocked and reared under polyculture method, along with Tilapia.. At the time of harvesting the average size of the fish was 740 g, (ranging from 300 to 1500 gms). The recovery rate was 68%The total expenditure was Rs.62,286 and the realization was Rs.2,37,000 with a profit of Rs.1,74,714/- under the polyculture method.

Advantage and disadvantage of Polyculture method

Advantages

1. Polyculture method is more natural

2. Fish can take feed depending upon the requirement

Since, the live fish only serve as feed, the water quality maintenance is easier. No contamination due to left over feed.

3. Cost effective.

Disadvantages

1. Not feasible at all places

2. The forage fishes like Tilapia itself are food fishes in some places. It supports cost effective fish for poor. The use of cheap fishes as food for growing the seabass will deprive the availability of fish for others. Since, the live fish won't be evenly distributed in the ponds,

3. Seabass having the chance to meet more fishes will feed more and grow large and many fishes will not have access for feed resulting in poor growth. This will increase the differential growth ultimately affecting the production returns.

Pond Culture of Seabass with Supplementary Feeding

Trash fish feeding

Seabass seed can be stocked in a prepared pond @ 10-20 thousand per ha. The seed size of 2.0 gm and above is preferable for stocking in the growout farms. Water depth should be maintained not less than 1.0 M. Seabass fishes stocked can be fed with minced meat of trash fish. Cheaper fishes like Tilapia, Sardines, horse mackerels which may not fetch more than Rs.5/- per kg can be bought from the commercial fish landing centers, washed and freezed in cold storages/Deep freezers as required. The fish can be taken out an hour/prior to feeding, thawed and minced as meat using meat mincer. Feed can be made as dough ball like paste and placed in trays, kept hanging in 4 or 5 places in the pond. Feeding rate is *ad libitum* in any case not more than 100% body weight on wet weight basis of the biomass initially and gradually reduced to 10% at last phase of culture period. Feed rations can be given in two doses in the Fore noon and After noon.

The feed quantity has to be adjusted according to the intake after checking in feed trays. A general guideline on the feed ration for wet fish feeding and compounded extruded floating pellet feeding rate are suggested in the Table.

Table : Schedule of Feeding

Culture Days	Rate of Feeding (Wet feeding like trash fish etc.,) %	Biomass % & Compounded pellet feed	Average size of the fish (in gms)
0-30	100	20	3-5
31-60	70	10	5-10
61-90	50	10	5-20
91-120	25	5	40-150
121-150	25	5	150-300
151-180	25	4	300-400
181-210	20	4	400-700
210-240	15	3	500-900
241-270	15	3	600-1200
271-300	10	3	700-1500

Feeding with Formulated Feed

Seabass is cultured feeding with extruded floating pellets in Australia, Thailand, Malaysia and Singapore. Being a carnivorous fish seabass needs high protein diet. Normally, in the preparation of diet for seabass, the animal ingredients are added more than 60% so that the required protein levels can be kept.

The Nutritional Requirement of the Seabass are as follows

Protein	:	around 55%
Lipid	:	15%
Fatty Acids	:	2%
Carbohydrates	:	15%

Since, Seabass is a fish feeding mainly on the fishes and shrimps moving in the water column (pelagic);the pellet should be slow sinking and should be in the column for reasonable time so that the fish can ingest the food before it reaches the bottom. For this extruded pellets are preferred. The extrude pellets will have reduced loss;the digestibility will be good due to pre cooking, the feed mixture can be with higher moisture, the flavor of feed also can be retained with addition of excess fish oil. The pellet size should be from 2.0 to 6.0 mm as per the size of the fish.

Pelleted feed for Seabass is manufactured by X1AMEN FWUSO Industry Co. Ltd., China with different sizes and stages of seabass. The grow out feed has 36-38 % protein;5-6%lipid, 15%Ash, 4-5%Fiber, 10%moisture;with feed pellet diameter 2 to 6 mm

Production and Economic Viability

Culture of seabass feeding with low cost fishes was carried out and the results are given below.

Feed trials conducted in the culture of Asian Seabass feeding with low cost fishes. From the commercial landings, low cost fishes like Oil sardine, horse mackerel etc were procured @Rs.3-6/kg as used as feed for seabass. The pond was stocked @2000/ha of average size 1.0 gm . After 11 moths culture period the fish grew to size of 517 gm to 1200 gms (average size of 946.5 gms). The recovery rate was 96%The production was in the order of 4.36 tons/ha. The realization was Rs.3, 61,585/- with expenditure of 3,14,800/- . The profit over the operational cost was to the tune of Rs.46, 785/-

Grow out Culture of Seabass in Cages

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

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

Design of Cages

Grow out cages of 20 or 50 M^2 are preferable for easy management and maintenance. Cages are fabricated with polyethylene netting with mesh size ranging from 2 to 8 cm depending upon juvenile fish propose to stocked in the cages. There are two types of cages

Floating net Cages

The net cages are attached to wooden frames kept afloat using plastic drums. Anchors or Concrete weight blocks as anchors can be attached to the corners of the net cage at the bottom. These types of cages can be installed in areas with water depth more than 4 meters with feeble water current.

Stationary net Cages

These are fixed enclosures, which can be installed, in shallow water areas in lagoons, brackishwater lakes having water depth of 2-4 meters. The cage net is fastened to wooden poles erected in the water system at the four corners.

Stocking Density

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

Feeding in Cages

Fishes in the cage can be fed with either extruded pellets or with low cost fishes as per the availability and cost. Floating pellets have advantages of procurement, storage and feeding. Since, a lot of low cost fishes are landed in the commercial landings in the coastal areas which are fetching around Rs.3-5/kg only used as feed for seabass culture. Low cost fishes like Tilapia available in the freshwater and brackishwater also serve as feed for seabass in ponds and in many cage culture operations. The rate of feeding can be maintained around 20% initially and reduced 10% and 5% gradually in the case of trash fish feeding and in the pellet feeding, the feeding rate can be around 5% initially and gradually reduced to 2-3% at later stage.

In the feeding of low cost fish FCR works out around 6 or 7 (i.e. 7 kgs of cheaper fishes has to be given for one kg of seabass). In the case pelleted feeding FCR is claimed to be around 1 to 1.2 in Australia. However, the cost effectiveness of the pellet feeding for seabass in grow out culture has to be tested.

Cage Management

Since cages are inside the water and exposed to water current, the debris materials drifted may adhere to the cages and clog the mesh restricting the water exchange. The fouling organism will also attach and clog the meshes. Other animals like Crab may damage the nets. The cages should be regularly checked for clogs and leaks. Damaged nets should be repaired or replaced. The clogging will reduce water exchange, and lead to accumulation of waste products depleting the oxygen causing stress to the fishes, affecting feeding and growth. If the damage is not repaired immediately, the fishes will escape from the cages.

Production

Under cage culture, since seabass can be intensively stocked and properly managed, the production will be high. Frequently culling and maintenance of uniform sized fishes in to the cages will ensure uniform growth and high production. Production of 6-8 kg/m^2 is possible in the cages, under normal maintenance and production as high as 20-25 kg/m^2 is obtained in intensive cage management in the culture of seabass.

Integration of Cage Culture of Seabass with Shrimp Culture

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

CONCEPT OF STRESS AND ITS MITIGATION IN AQUACULTURE

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Introduction

Increasing population has tremendously increased pressure on fisheries and aquaculture to increase the fish production. To increase the production intensification of aquaculture became very essential. In intensive aquaculture practices animals comes across the various types of physical, chemical and biological stress under poor management practice, which ultimate lead to failure of aquaculture. Hence stress management is an important aspect in aquaculture practices.

Different stressors such as physical, chemical, biological and procedural exist in different stages of aquaculture practices. The physiological responses (stress response) of the animals exposed to stressor are of three types i.e. primary response, secondary response and tertiary response (Wedemeyer and Mc Leay, 1981).

It is generally accepted that the animal responds to a variety of stressors, which is an adaptive mechanism and called as general adaptation syndrome (GAS) (Barton and Iwama, 1991).

There are two approaches to mitigate the stress first one is non-chemical (biological method) and the other one is the chemical method. Non-chemical (biological) method includes the entire environment management which includes water quality management such as temperature, dissolved oxygen, ammonia, nitrogen, nitrite, salinity etc. and stocking density, uniform size stocking, stocking ratio in polyculture etc. Chemical method includes dietary supplementation of vitamin C, Vitamin E, tryptophan, immunostimulants etc.

Concept of stress and stressors

Stress

Stress was defined by Seyle (1950): Stress means the sum of all the physiological response by which an animal tries to maintain or re-establish a normal metabolism on the face of physical or chemical force.

Stressors

Factor that causes stress is called stressors. Rearing of aquatic organisms in man made environment has resulted in exposure to a number of stressors, which may not be experienced to the same degree in natural environments. Stressors in aquaculture systems may be categorized as below: (Wedemeyer, 1999)

Table 1: Type of Stressors and its examples

Sr. No.	Type of Stress	Examples
1.	Physical	Temperature, Light, Dissolved oxygen, Sound
2.	Chemical	Water quality, Pollution, Diet, Metabolic waste
3.	Biological	Stocking density, Microorganisms (pathogenic and non-pathogenic), Macro organisms (parasites), Lateral swimming space requirements
4.	Procedural	Handling, Hauling, Stocking, Disease treatment, Feeding methods (manual and automated)

Stress responses

Fishes are ectothermic organisms and therefore are unable to control their body temperature within narrow temperature limits (Hazel, 1993). As a result, their physiology is dependent on ambient temperature. The environmental temperature determines the metabolic rate, physiology and growth of fishes (Morgan *et al.*, 1999). Water temperature influences O_2 concentration, metabolism and growth of fishes (Langston *et al.*, 2002).

Stressor is causative factor and stress is a response. The stress response of fish follows the general vertebrate pattern. A key element in the stress response is a switch from anabolism to catabolism. The quantum of response may vary with nature of stress and its duration, and it also dependents on factors like age, sex, maturation stage, species and strain of fish.

When fish and other aquatic organisms experience environmental disturbances that lie outside the normal range, the effect may be dramatic. At the organism level, a series of physiological changes occur following stressful challenges, which are adaptive in nature. These physiological responses are termed as **'General Adaptation Syndrome'** (GAS), Selye, 1950. It is consist of:

(1) An *alarm reaction* in which "stress hormones" (catecholamine and corticosteroids) are released.

(2) A *stage of resistance* during which adaptation occurs.

(3) A *stage of exhaustion* in which adaptation is lost because the stress was too severe or long lasting.

Stress response has been classified into primary (neural and neuro-endocrine responses), secondary (physiological consequence of such primary response) and tertiary response (changes in behaviour, growth rate, increased susceptibility to diseases and change in population) (Wedemayer and Mc Leay (1981). Primary stress response results in the activation of the neuro –endocrine system, which brings about changes in metabolism, osmoregulation and haematology (Barton, 2000). The primary stress response is the physiological alarm, during which there is an increase in the levels of "stress hormones" i.e. corticosteroids and catecholamines. These primary responses result in a suite of biochemical and physiological changes leading to secondary stress response, characterised by elevated glucose levels, haemato-immunological changes and change in other metabolic enzymes. Secondary stress responses are believed to be adaptive mechanisms and are particularly important for fish to recover from stress. However, during chronic conditions the fish loses its capacity to adapt. This results in pathological changes, reduction in reproductive success, decrease in growth rate and decreased disease resistance that leads to death of the organism thereby causing change in the population and biodiversity of that species, which is termed as tertiary stress response.

Primary stress responses

I. Release of adrenocorticotropic hormones (ACTH) from the adenohypophysis.

II. Release of "stress hormones" (Catecholemines i.e. adrenalin, nor-adrenalin and dopamine and Corticosteroids especially cortisol) from the head kidney.

Sensory perception of stress is a prerequisite for stress response in animals. In fish, an adverse condition stimulates the afferent neural pathway that run in the sympathetic nervous system from the hypothalamus to the chromaffin tissue of the head of kidney and stimulates the chromaffin tissue, which leads to the release of

catecholamines. Catecholamine (Adrenalin / Epinephrine) is released from the chromaffin tissue in the head kidney of teleosts, and also from the ending of adrenergic nerves. Because catecholamines, predominantly epinephrine in teleostean fishes are stored in chromaffin cells, their release is rapid and the circulating levels of these hormones increase immediately with stress. The release of catecholamines is extremely rapid compared to the release of cortisol.

Corticotropin-releasing hormone (CRH) or factor (CRF), released from hypothalamus of brain, which stimulates corticotrophic cells of anterior pituitary (adenohypophysis) to secrete adrenocorticotropic hormone (ACTH), which stimulates interrenal cells (adrenal cortex homologue) to synthesize and release corticosteroids particularly cortisol which is the principal corticosteroid in fish. The release of cortisol in teleost is delayed relative to catecholamine release. Resting and unstressed levels of circulating corticosteroids in fish are less than 30-40 ng / ml (Wedemeyer *et al.*, 1990). Characteristic cortisol elevation of fishes in response to acute stressors tends to range within about 30 to 300 ng / ml (Wedemeyer *et al.*, 1990; Barton and Iwama, 1991). Elevation of plasma catecholamines and cortisol due to primary stress lead to secondary stress responses.

Secondary stress responses

In fish, cortisol enters liver cells where it binds to nuclear receptor, resulting in activation of genes that produce a series of enzymes that have a range of metabolic effects. This results in a suite of biochemical and physiological changes which may include hyperglycemia, hyperlacticaemia , depletion of tissue glycogen reserves, lipolysis and inhibition of protein synthesis. Other changes may include the osmotic and ionic disturbances due to diuresis and loss of electrolyte from the blood and change in haematology (reduction of white blood cells, leucopenia). (Barton and Iwama, 1991). Stress is an energy demanding process and the animal mobilizes energy substrate to cope with stress metabolically. The production of glucose under stress assists animals to cope with the increased energy demand. The stress hormones adrenaline and cortisol have been shown to increase plasma glucose production in fish by both gluconeogenesis and glycogenolysis.

Catecholamines in particular, have marked influence on cardiovascular functions leading to change of blood circulation, gill perfusion and oxygen carrying capacity of blood. Corticosteroids on the other hand are known to stimulate the ion-transport mechanism in the gill and kidney.

These secondary stress responses are believed to be adaptive mechanisms and are particularly important for fish to recover from stress by maintaining oxygen supply to the tissues, to regain osmotic and ionic equilibrium and to meet the increased energy demands imposed by exposure to environmental stressor. Typically these changes persists only for few hours or days following acute exposure to the stressor and therefore do not result in any deleterious effect on the animal.

Intracellular stress response characterized by the production of a family of proteins known as heat shock proteins (HSP). Exposure of cells or whole organisms to heat shock results in a reversible increase in the synthesis of some acute phase proteins against subsequent shock known as HSP (Palmisano *et al.*, 2000; Ming *et al.*, 2003), which play an important role in homeostasis. HSPs are a family of highly conserved cellular proteins that have been observed in all organisms (Feder and Hofmann, 1999) including fish (Iwama et al., 1998). They were first discovered in the chromosomal puffs of drosophila salivary glands after thermal shock (Ritossa, 1962). In the normal unstressed cells heat shock proteins are essential for folding and translocation of newly formed proteins and renaturation of denatured proteins. The expression of these proteins increases manifolds in the cells during stress. HSP has an ability to mediate misfolded or denatured functional proteins caused by various stressors in the cell hence this protein is also known as molecular chaperone.

Tertiary stress responses

Chronic exposure to stressors provokes tertiary stress responses that result in a number of pathological changes and reduction in reproductive success, depression of growth rate and decreased disease resistance. Tertiary stress response represents whole animal and population level changes associated with stress.

i. Whole animal:
   a. Impaired growth, Parr-smolt transformation (smelting), spawning success and migration behaviour and spawning.
   b. Increased disease incidence (infectious and noninfectious)

ii. Population parameters:
   a. Reduced intrinsic growth rate, recruitment, compensatory reserve and productivity.
   b. Altered community species abundance and diversity.

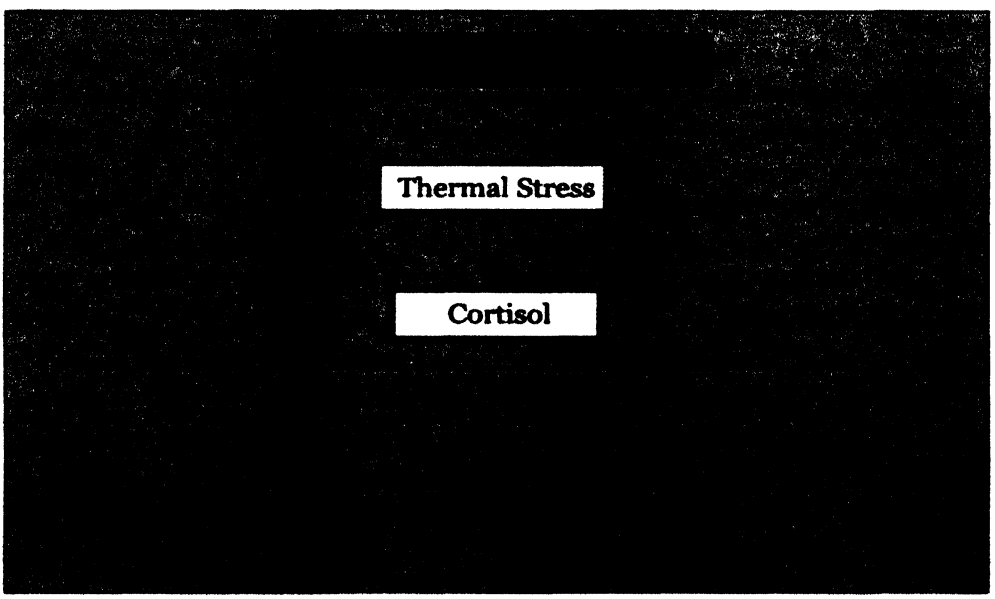
Thus, when fish are exposed to environmental stressor, a hierarchy of responses is initiated and if the stress is severe or long lasting, successively higher levels of

biological organisation gets affected. This signifies that the primary responses are the changes at the endocrine level; where as the tertiary responses refer to those changes that can be easily seen by observing the animal.

Stress mitigation methods

One of the most promising areas of research is the development of strategies to reduce the stress during various aquaculture practices. There are two approaches to mitigate stress, first one is non-chemical (biological method) and other is chemical method. Non-chemical (biological) method includes the entire environmental management, which include water quality management such as: temperature, dissolved oxygen, ammonia, nitrogen, nitrite, salinity etc. and optimum stocking density, uniform size stocking, stocking ratio in polyculture etc. Chemical method includes dietary supplementation of vitamin C, Vitamin E and tryptophan and immunostimulants *etc.*

Flow Diagram: Stress Mitigation Measures



1. Non-chemical methods (Environmental management)

Water quality is defined as the suitability of water for the survival and growth of fishes where as water quality management is a technique to bring the water quality in desirable level for the growth and survival of fish. Environment management in

aquaculture means water quality management such as dissolve oxygen (DO), water temperature, alkalinity, water Ph, turbidity, hardness, etc.

Table: Showing some of the important water quality parameters and their optimum range for fish culture.

Sl. No.	Water quality parameters	Optimum range
1	Dissolved Oxygen	>5 ppm
2	Temperature	28 to 31 ^{0}C
3	Turbidity	20 to 60 cm
4	Total hardness	40 to 100 ppm
5	Total alkalinity	60 to 300 ppm
6	Ph	7.0 to 7.5
7.	NH_3-N	<0.05

2. Chemical methods

I. Dietary supplementation of protein, vitamin C & vitamin E

Oxidative stress is caused by the production of reactive oxygen species and nitrogen species during stress (Gordon, 2000). Oxygen radicals is generated due to respiratory burst activity of phagocytes, present in cells under normal conditions but its production increases during pathophysiological conditions and stress. The use of immunostimulants and antioxidants to ameliorate the damage to immune system by stress has been studied by many workers. Fishes fed with immunostimulant glucan prior to transportation have increased specific and non specific immune response as is evident by higher number of lymphocytes and enhanced phagocytosis (Jeney *et al.*, 1997).

It is reported that supplementation of high protein and vitamin C reduced the bioaccumulation and stress responses in *Channa punctatus* (Sarma, 2004). Dietary high protein and vitamin C were supplemented for ameliorating stress (Manush *et al.*, 2005) in *Macrobrachium rosenbergii*. Vitamin C is considered to play an important role in animal health as antioxidants inactivate damage of free radicals produced during normal cellular activity from various stressors have been reported confirming protective role of Vitamin C. Vitamin C can interact with other antioxidants such as carotenoids and vitamin E. A high concentration of vitamin C at the cell membrane regenerates the reduced vitamin E created during oxidation and reduction process. Vitamin C is highly interactive and may fortify antioxidant defenses and enhance immune response indirectly by maintaining optimal Vitamin E levels. Vitamin C and vitamin E acts as membranes protecting agents and give stability to lipid bilayer.

II. Dietary supplementation of L-tryptophan

Tryptophan is one of the eight essential amino acids, necessary for the development of the vitamin niacin / nicotinic acid and serotonin. It cannot be synthesized in the body and thus must be obtained from food or supplements. L-

tryptophan act as cortisol blocker hence reduces stress induced production of plasma cortisol.

iii. Dietary supplementation of Lactoferin

Lactoferin (LF) is a family of iron binding glycoproteins having molecular weight of 80 KDa that originated from some secretion of mammals. It is a kind of immunostimulant and has a lot of biological functions e.g., Iron absorption and transportation, bacteriostatic effects and enhancement of mucosal immunity system in mammals. Orally administration of LF enhances nonspecific defense system and phagocytic activity in rainbow trout and decrease plasma cortisol level in gold fish by I.F administration. Dietary LF also enhances tolerance to physiological stressors such as air exposed stress in juvenile Japanese flounder against high stocking density stress in rainbow trout and common carp and low salinity stress in shrimp (Koshio *et. al.*, 2000).

Conclusion

During aquaculture practices animals come across many different types of stressors such as physical, chemical and biological. To cope up with these stressors physico-biochemical process of biomolecules, cells, organelles and organisms vary from species to species. Exposure of fishes to extreme environmental conditions elicits a cascade of physiological and biochemical changes characterised as primary, secondary and tertiary stress responses. Stress can be mitigated by biological and chemical methods.

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TRADITIONAL FARMING OF SEABASS IN WEST BENGAL

G. Biswas

1. Introduction

The traditional methods of fish/shrimp farming have been practised in West Bengal since 18th Century. This system is known as "Bhasa Badha Fisheries" and the culture impoundments are called as *Bheries*. In this system fishes and shrimps are allowed to grow together in polyculture mode. Among the fishes farmed in this system, Asian seabass, *Lates calcarifer* is the most priced one due to its high market demand. Having staple market and other biological characteristics like euryhaline nature, fast growth rate etc., the farmers consider it as the most lucrative species for brackishwater aquaculture diversification other than shrimp.

2. Brackishwater resource potential of West Bengal

West Bengal has three coastal districts, *viz*. North and South 24 Parganas and East Midnapore spreading over a short coast line of about 158 km. The state has total estimated potential brackishwater area of around 4.05 lakh ha (34% of country's potential), out of which 2.1 lakh ha found suitable for coastal aquaculture. Till recent past total area of 50,438 ha has been brought under brackishwater farming with main produce as shrimp of 14,483 tonnes during 2006-07. Brackishwater Fish Farmer's Development Agency (BFDA) located at each coastal district implements different government schemes for promotion of brackishwater aquaculture. Total 5300 ha brackishwater area has been brought under different BFDA schemes till 2006-07. In West Bengal, more than 80% of the brackishwater fishery belongs to traditional type of farming with trap and culture system, locally known as *bheries*. These *bheries* are located mainly in the low-lying areas of North and South 24 Parganas districts.

3. Seabass seed availability and seed trade

Natural seabass seeds become available in tidal creeks and intertidal pools during May-October in the Hooghly-Matlah estuarine system in West Bengal. Intertidal pools with dense grass vegetation form the main source of collection areas. Hapa net is commonly operated in sheltered areas at the end of high tide, while shooting nets are operated during high tide in the intertidal zones for capturing the wild seeds.

Seabass seeds are collected from different locations of Sunderbans such as Kakdwip, Namkhana, Bakkhali, Sagar Island etc. by fishers (seed collectors). These collected seeds are brought to fish seed markets located at Nishchintapur, Chemaguri, Harwood Point (Lot no.8) in South 24 Parganas district. Nishchintapur is the most important brackishwater fish seed market in South 24 Parganas. There are two prime market days (Hat bar) on Sunday and Thursday in a week at Nishchintapur. Price of seed ranges from Re.1-7/- per piece depending on size variability over a period of 6 months at Nishchintapur market in the following manner.

May: Early fry (10 mm) - Re.1
June: Fry (20 mm) - Rs.2
July: Advanced fry (40-45 mm) - Rs.3
August: Fingerling (55-60 mm) - Rs.4
September and October: Advanced fingerling (90-130 mm) - Rs.5-7
Middlemen are very often involved in the seed trades. The seed market chains existing here are:
Seed collector → Wholesaler → Retailer → Farmer
Seed collector → Wholesaler → Farmer
Seed collector → Wholesaler → Seed exporter → Farmer
Seed collector → Farmer

Maximum quantity (80%) of seabass seeds from this market are sent to North 24 Parganas district in the *bhery* areas through middlemen. From there some quantity is also exported to Bangladesh. Around 10% of the total seabass seeds brought in the market are purchased by local farmers. And the rest 10% go to other states like Orissa and some places of Andhra Pradesh.

4. Types of culture

Traditional culture of fishes in *bheries* is an age old practice here. Earlier these *bheries* were stocked with the natural seeds entered through tide only and allowed to grow at the mercy of natural productivity. The availability of seeds from natural sources has already become uncertain and dwindling for several reasons. So the *bhery* farmers have modified their culture practices accordingly by following selective stocking, fertilization, feeding etc. In this process seabass is also stocked in *bheries* as a selected species based on availability of seeds. Some progressive farmers also follow improved culture practices like monoculture and polyculture of seabass in their ponds when they get required number of stocking materials.

4.1 Traditional culture of seabass

Brackishwater tidal wetlands, namely mudflats, swamps, marshes, paddy fields etc., located in low-lying areas of North and South 24 Parganas districts of West Bengal, in which fisheries are developed, are locally known as *bheries*. These are of irregular shaped and their sizes range from 2 to 267 ha with the average size of 15-37 ha spreading over low, medium and high salinity zones. The estuaries of Saptamukhi, Thakuran and Matlah (under Hooghly-Matlah estuarine system) and other minor estuaries like Gosaba, Muriganga, Haribhanga, Kulti, Ichamati, Raimangal etc., with their tributaries and distributaries feed the *bheries* with their tidal waters. There are two types of *bheries*, seasonal and perennial. In the seasonal *bheries* both fish/shrimp and paddy are raised either through simultaneous or rotational systems, whereas in the perennial bheries fish/shrimp are raised almost throughout the year. Polyculture systems are practised in *bheries* with large number of fish and shrimp seeds brought in through the tidal water and partial stocking. Fish culture operations start from February and continue till April. Large scale harvesting in the *bheries* is done between September and November. The important fishes occurred in the bheries include *L. calcarifer*, two species of mullets, *Liza tade*, *L. parsia*, cat fish, *Mystus gulio*, *Elops spp.*, *Megalops cyprinoides*,

Eleutheronema tetradactylum, *Therapon jarbua*, *Glossogobius giuris* etc., while the shrimps trapped consist of *Penaeus monodon*, *Fenneropenaeus indicus*, *Metapenaeus monoceros*, *M. brevicornis*, prawns, *Macrobrachium rosenbergii*, *M. rude*, *M. malcolmsonii*, *Palaemon styliferus* etc. and crab, *Scylla serrata*. Now-a-days, supplementary stocking of selected fish species, such as *L. calcarifer*, *L. tade*, *L. parsia*, *Mugil cephalus*, *Oreochromis mossambicus*, *O. niloticus* is followed but in most cases the stock is left at the mercy of nature and the predators. Supplementary feeding is not generally practiced as the entire production system is dependent on utilization of natural productivity of *bheries*. However, some framers use oil cake and rice bran as supplementary feed.

At present with the regular occurrence of white spot disease in shrimp, *bhery* farmers are opting some eco-friendly and sustainable culture systems mainly comprising of finfishes. For this purpose they have selected Bhetki as a candidate species and are culturing it in polyculture system. They do not follow any specific stocking rate due to uncertain availability of natural seeds. Sometimes juveniles of Tilapia serve as prey to seabass. Harvesting is done after 6-8 months of culture and large sized seabass are found with less in numbers compared to improved culture systems. In this system production ranges from 500-1000 kg/ha/year.

4.2 Improved culture methods

Seed is a critical input and when it is available in sufficient quantity farmers go for improved culture practices. There are two culture systems followed, *viz.* polyculture in predatory prey system and monoculture. In polyculture method, seabass is grown alongwith some other fish species which serve as food for seabass. Here the prey fishes should be prolific breeder to produce sufficient numbers of offsprings or their juveniles should be stocked at regular interval. Whereas, in monoculture system the stock is totally raised on supplementary feed. Stocked seabass are fed with minced flesh of cheaper trash fishes.

5. Constraints affecting transition from traditional to improved methods

There are a number of constraints affecting transition from traditional to improved culture methods of seabass in the state, which are to be addressed. The problems can be grouped into four categories.

5.1 Input related

(i) Seed: The major problem in this sector is unavailability of quality seeds in adequate quantity at appropriate time. Presently the culture developed in whatever extent in *bheries* and ponds depends totally on natural seeds. The unpredicted availability of natural seeds with size variability affects the grow-out culture. The price of the seeds is also high. When monoculture is undertaken with natural seeds, the farmers face difficulty in weaning them to dead trash fish or minced meat. There will not be this problem with hatchery produced seeds which are weaned to supplementary feeds prior to stocking.

Artificial seed production technology developed by CIBA would address this problem after adoption by entrepreneurs.

(ii) Feed: Another important constraint directly affecting the expansion of seabass culture is lack of availability of formulated feed and inadequate or stray supply of trash fish. Seabass feed should have high protein content. Supply of trash fish is seasonal and also insufficient in quantity. Availability of trash fish is poor with high price due to high demand of fish meal produced from it. Also trash fish itself is a staple diet of the poor of coastal areas in developing country like India. It requires purchase in bulk amount and storing in deep freezer. So the small and marginal farmers who own around 50% of cultured brackishwater area can not afford this. In polyculture, farmers face problems getting live forage fish in sufficient quantity. Development of suitable slow sinking formulated feed can only address these issues.

CIBA has already developed nursery feed for seabass and efforts are on for larval and grow-out feed development. Some foreign firms have entered seabass feed supply system, but the economic feasibility of these feeds is still a major concern for development of seabass farming in India.

(iii) Supply of basic inputs: Basic inputs for seabass farming, *viz*. seed, feed, lime, fertilizers etc. are supplied almost in unorganized way. Farmers are very often misled by the local agents, dealers supplying those materials.

5.2 Technical/ managerial

As the culture of seabass is not a developed sector compared to shrimp farming, the farmers are ignorant about basic biology like feeding habit, growth, survival etc. Seabass juveniles prefer moving live food, so during culture with wild seeds, training the fish to accept trash fish or artificial feed (weaning) requires some experiences and skills. Prior to feeding, sometimes fishes are attracted by producing sound in water and then the chopped trash fish or formulated feed is broadcasted slowly, so that the fish would notice the sinking object as a moving prey and engulf them. The fish are fed at the same time everyday. Differential growth in the same age group is another important inherent quality which needs management attention. The individuals having a minimum size difference of 33% from the rest of the stock are called 'shooters'. These shooters cannibal on the smaller ones leading to poor survival and production. It is managed by segregating them periodically and culturing separately.

5.3 Financial and policy

Seabass culture requires higher capital investment due to costs of seeds, feeds etc. Small farmers are mainly dependent on local money lenders who take very high rate of interests. For this constraint farmers sometimes think a while prior to undertake seabass culture. There are many schemes for development of freshwater fish culture as well as shrimp farming ventures sponsored by the State Fisheries Department, Fish Farmer's Development Agency and BFDA, but no scheme exists on promotion of seabass culture at present. As a result, the State Fisheries Department as the main extension functionary can not provide proper guidance to farmers also. No attempts were made to set up seabass hatchery by any private or Govt. firms in the state. CIBA has already developed the year round seabass seed production technology. Private entrepreneurs and Govt. agencies can solve the seed supply problem after adopting this technology.

5.4 Environmental

(i) Natural calamities: Heavy monsoon rainfalls sometimes cause floods resulting in the total loss of fish crop. Due to this, farmers sometimes show their reluctance to take this high investment culture.

(ii) Pollution: The *bheries* which are the important resources for seabass culture, presently witness pollution problem due to sewage effluent discharge from Kolkata Metropolitan City. It is adversely affecting the production in *bhery* system.

5.5 Social

Seabass farming is gaining popularity among both freshwater and brackishwater farmers. In earlier days seabass catch was mainly from brackishwater estuarine and *bhery* systems and farmers had the idea that it is a saline water fish. But with the accelerated domestic market demand freshwater aquaculturists are showing interests gradually towards this farming and becoming successful. In brackishwater, the expansion of seabass culture is in slow pace due to some input related bottlenecks. Though there is regular occurrence of white spot disease in shrimp, farmers are still opting for shrimp culture in traditional water bodies with the hope to earn more profit within a short period and consequently facing huge loss. There is need to break this jinx and change their mind-set for not to utilize the farm wholly for shrimp culture and to undertake finfish culture in some ponds for their sustenance.

6. Culture expansion possibilities

Due to problems of environmental degradation and disease incidence in shrimp farming in brackishwater systems, culture of seabass as an alternative for diversification has immense scope without degrading the environment to any significant extent. Farmers are slowly turning to finfish culture and *L. calcarifer* is the most lucrative species of their choice. As West Bengal has a good domestic market, this fish does not face any marketing problem. The traditional *bhery* farming system does not ensure any steady and substantial yield, so the farmers have already undertaken seabass culture for their sustenance. The abandoned shrimp farms can well be utilized by culturing seabass there. In freshwater sector, the market price for Indian major carps and other carps has become almost static and the margin of return is reducing with the regular increase in production cost. So, culture of some high-price, high-demand fish like seabass will definitely solve their problems as it can grow in freshwater also. This species has also good export potential in the form of processed items. Different processing plants are showing their interests in export-oriented seabass products development. Although seabass culture has not yet developed into a commercial scale in West Bengal, it is receiving considerable attention, particularly by a number of farmers, research institutes, processing plants.

7. Conclusion

With the availability of seed production technology, the time has come to mass scale production of suitable stocking materials by different entrepreneurs adopting this technology. As seed is the most important primary input, when it will be available at

required level to the farmers, there is scope to modify the traditional culture process into improved one. To meet up the increasing demand for fish, it is more pertinent at present scenario, to switch over from traditional farming to at least modified traditional or improved culture systems. In this direction the technologies developed so far for seabass breeding and culture will be of significant use to the end users.

POND BASED NURSERY REARING OF SEABASS

G. Biswas& J K Sundaray

1. Introduction

The availability of fish seed is considered to be one of the most important factors for the success of seabass culture in any water body. Till recently for the practice of seabass culture in India, the fish farmers have to depend on collection of seabass fry or fingerlings from the wild and then stock them in ponds or cages. The availability of fish seed varies considerably from year to year and the uncertain supply of this input results in limited seabass culture. With the development of technology package for artificial propagation and year round mass scale production of seabass fry by CIBA, it is anticipated that the much awaited seed demand would be met up by interested entrepreneurs after adoption of this technology. Seabass larvae grown for 25-30 days in hatchery are reared further for a period of 30-45 day nursery phase till they attain suitable size for stocking in grow-out culture systems. Generally, seabass nursery is carried out in fertilized brackishwater ponds and in net cages placed inside a pond or in an open coastal area. Here different types of pond based rearing systems are described.

2. Nursery rearing

Seabass fry is highly carnivorous and voracious feeder and development of shooters drastically reduces the survival percentage through cannibalism. From a management standpoint, priority must be given to control the cannibalistic behaviour which is one of the most important causes of mortality in nursery. So during designing any nursery system due consideration must be given towards manageability of these causes of mortality. Two pond based nursery rearing systems are followed for seabass.

2.1 Nursery rearing in pond

Ponds used for nursery rearing should be not more than 2000 m^2 for easy management with most preferred size of 200-500 m^2 holding at least 70-80 cm water. Ponds should have the provision of inlet and outlet fitted with small mesh net. Ponds are prepared and fertilized to eradicate predators and grow zooplankton at least two weeks prior to stocking. When the pond water is with natural algae growth, freshly hatched Artemia nauplii are introduced. Usually, for 1 ha pond 1 kg cyst is required. Seabass fry acclimatized to pond condition is stocked @ 20-30 nos/m^2. At least 30% water is exchanged daily. Supplementary feeding is done with chopped, cooked fish/shrimp meat 3 times daily @ 100% body weight in the 1st week followed by gradually reduced to 80, 60, 40 and 20% during 2nd, 3rd, 4th and 5th week, respectively. Excess feeding should always be avoided to maintain the optimum pond environment. At the end of rearing around 40-50% survival is achieved with 3-5 g body weight.

2.2 Nursery rearing in net cages (hapas)

Seabass fry is reared in net cages or hapas fixed in ponds. Hapas are either rectangular or square shaped enclosures made of nylon thread webbing and are kept inverted with top portion open.

2.2.1 Pond preparation and hapa arrangement

The pond where hapas are to be fixed are prepared well following weed eradication, liming etc. to maintain desirable water condition one week prior to start of rearing. Mosquito net hapas made with 1/8-inch nylon webbing of size 1x1x1.5 or 1x1x2 m^3 are fixed with bamboo poles in both sides of a catwalk in the pond by keeping 20 cm free board. The catwalk is erected to facilitate feeding, hapa checking, grading and other management activities. Sometimes use of double-layered hapas are suggested where chance of breaching of hapa wall by crabs or any other animals is high to prevent escape of fry under rearing.

2.2.2 Stocking

Healthy fry with 1 to 1.5 cm size are stocked @200-500 nos./m^2 after proper acclimatization during cool hours of day. Seeds are counted and distributed to each hapa. Uniform size fry are stocked. Initial biomass is calculated by taking samples and it is required for feed calculation.

2.2.3 Feeding

The fry are fed either with slow sinking formulated feed or minced meat 3 times daily during day hours. Formulated feed is provided @10-5% body weight daily throughout the rearing period, whereas for minced meat feeding the rate is similar to that of pond rearing system. Feed amount is adjusted weekly from sampling data of growth increment.

2.2.4 Grading of fry/ separation of shooters

To get higher survival it is needed to sort and size-grade shooters regularly to lower competition for space and food, thus controlling cannibalism. Shooters are the individuals which have a minimum size difference of approximately 33% from the rest of the stock and they are removed weekly by hand picking from each hapa. These shooters are reared separately.

2.2.5 Hapa management

Net cages (hapa) provide almost natural condition for the growing fishes. There are chances of the mesh get clogged due to adherence of debris or weeds and thus restricting water movement and resulting in stagnation of water and accumulation of waste products and algal growth in the hapas. Hapas are cleaned at 2-3 day intervals to avoid this problem. At the same time the hapas should be checked for any damage by crabs.

2.2.6 Growth and survival

Growth and survival depend on the stocking density, feed and feeding, shooter separation, etc. If all management activities undertaken properly, in 4-6 weeks, the fry attain 50-70 mm size with 2-3 g body weight and survival rate upto 80% is achieved.

2.2.7 Advantages of hapa rearing

There are a number of advantages of hapa nursery rearing compared to other nursery rearing methods. These are, i) hapas can be easily managed and require less space and capital investment, ii) farmer can extend it to any scale depending on his necessity and capability, iii) it can be maintained in a corner of grow-out pond or near the grow-out cages itself, iv) since cages or hapas are in in situ condition, it provides natural environmental condition, v) water flow in the cage site washes away the metabolites and uneaten feed, vi) growth and survival are higher in hapa rearing, vii) total harvesting of seed is possible.

3. Causes of mortalities in nursery rearing

There are some reasons which are directly or indirectly involved in mortalities during nursery rearing.

3.1 Cannibalism

Seabass is highly cannibalistic especially in the early life stages. The cannibalistic behaviour of this species is definitely one of the major causes of high nursery mortality, it would appear to be dependent upon a number of factors. Cannibalistic rate increases with increasing stocking density, water transparency and decreasing light intensity, number of feeding per day, etc.

3.2 Stocking density

High stocking density is another common cause of nursery mortality especially in the absence of stock management measures. As stocking density increases, the percentage of mortality accordingly increases for the same culture period.

3.3 Differential growth

Differential or uneven growth of the same stock is a common phenomenon in seabass throughout the life period. It promotes competition among the individuals for feed, space and other essentials for survival. The resulting additive effects of stresses on the smaller and weaker fry make them dark to black colour, posing them much more susceptible to being preyed and diseases. This uneven growth may also be attributed to dietary and environmental factors.

3.4 Disease infection

Disease infection has been known to be responsible for mass mortalities of fry in seabass nursery operations. The causative agents for the diseases are generally

referred to viruses, bacteria, fungi, protozoans and other harmful pathogens including helminths. In the case of seabass disease infection the culturists pay proper attention until it is in advanced stages when the symptoms are more easily discernible, but by that time treatment becomes ineffective. There are some treatments like immersion in formalin or other antimicrobial substances. Although these external treatments have certain positive effect on the sick fish, but from practical application point of view, they may not be suitable for mass scale administer under cage and pond conditions. So the only way to prevent these diseases is maintenance of optimum culture environment.

4. Conclusion

Nursery rearing of seabass fry is an important phase to get suitable size stocking materials for grow-out farming, as stocking bigger size seeds directly increases survival and production. It is an important intermediate stage of rearing between hatchery and grow-out phase. Among the two pond based rearing systems discussed here, rearing in net cages (hapa) is more advantageous over direct pond rearing in terms of management and output point of views. Farmers should take up this type of rearing before stocking in culture ponds.

SOIL AND WATER QUALITY REQUIREMENTS AND THEIR MANAGEMENT IN BRACKISHWATER AQUACULTURE

R.Saraswathy, M. Muralidhar, K.K. Kirshnani and B.P. Gupta

Introduction

Successful aquaculture depends on providing animals with a satisfactory environment in which to grow. The most important principle regarding soil and water quality management is that a pond has a finite capacity to assimilate nutrients and organic matter. When the capacity is exceeded, water and soil quality will deteriorate. Maintaining a good culture environment through use of proper management practices will reduce the risk of disease and increase production, productivity and marketability.

Soils

Soils are a major factor in pond aquaculture, because ponds are made of soil material and the condition of pond bottom influences water quality and production. Concentrations of nutrients and phytoplankton productivity in pond water are related to pH, 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

Soil texture refers to the relative percentage of sand, silt and clay in the soil. It has direct effect on the productivity of ponds. Clayey soils are best suited for constructing bunds and have good water retention properties. Sandy soil is porous and very poor material for constructing bunds. Therefore Brackishwater soils with moderately heavy texture such as sandy clay, sandy clay loam and clay loam are highly suitable for aquaculture.

pH

pH gives an idea whether the soil is acidic or alkaline. It is an important parameter and it influences availability of nutrients, rate of mineralization, bacterial activities and fixation of phosphorus. In general soil pH ranging between 6.5 and 7.5 are best suited for Brackishwater environment. Under this pH range, the availability of nitrogen, phosphorus, potassium, sulfur, calcium and magnesium concentration is maximum.

Calcium carbonate

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. It decreases BOD and enhances nitrification due to the requirement of calcium by nitrifying organisms. The productive soil should have calcium carbonate more than 5%.

Organic matter

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%.

Pond soil management:

Successful aquaculture depends on good bottom soil condition. On the other hand, even if the site is good, problems may still crop up by the large quantity of inputs like feed and fertilizers which lead to excessive phytoplankton production, low DO, high ammonia, poor bottom soil condition and other problems. Most of these problems can be avoided by proper management practices such as site selection, pond preparation, liming, moderate stocking etc. at three stages viz. Newly Pond construction, Pond preparation after harvest and during culture period.

1. Newly constructed ponds

In newly dug out ponds, understanding of the soil parameters helps to decide the management strategies to be followed in terms of liming, manuring, fertilization, water management etc.,

2. Pond preparation after harvest

Before initiating second crop, the pond has to be prepared for stocking. Following are the importance practices to be followed

Cleaning:

Waste accumulated during culture must be removed by draining and drying of pond bottom to ensure sustained production in pond.

Pond mud drying and sediment removal:

After draining the water, pond bottom is sun dried for atleast 7-10 days and the soil should crack to a depth of 25-50 mm. After drying, the waste can either be removed manually or with machines. It makes pond bottom harder and it may reduce the levels of some pathogens in the pond. It also enhances aeration and microbial decomposition of organic matter. In aerobic decomposition, organic matter is oxidized to inorganic substances such as CO_2, water, ammonia, sulphate, phosphate, etc.

Tilling:

After the crop is harvested, undesirable species like pests, competitors and predators remaining in the ponds can be removed by physical (drying of ponds) and chemical methods (application of Mahua oil cake & tea seed cake).

Liming:

Liming is most important in pond preparation to keep the pond environment hygienic for sustainable fish production. It increases pH of soil, enhances availability of nutrients, accelerates microbial activity, maintains alkalinity and increases availability of CO_2. It also improves the hygiene of the pond bottom, permits normal reproduction and growth, thereby improving survival of aquaculture species.

3. Management of pond bottom during culture

During culture, the uneaten feed by the aquatic animals sinks to pond bottom. The carbonaceous matter, suspended solids, faecal matter and dead plankton also settle at the pond bottom. These materials have combined effect on the environment of pond bottom. To understand the condition of pond bottom, the following parameters are to be monitored regularly.

pH of soil:

This is one of the most important soil quality parameters since it affects the pond condition. Generally, soil pH ranging between 6.5 and 7.5 is the best suited where availability of macro nutrients are maximum. The low pH of bottom sediment indicates unhygienic condition and needs regular check up.

Redox-potential:

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. Negative redox value shows reducing condition whereas positive value shows aerobic condition. Under anaerobic condition of the pond bottom, reduced substances such as H_2S, NH_3, CH_4 etc. is formed which are toxic to benthic organisms.

Water circulation by water exchange, wind or aeration helps to move water across mud surface and prevent the development of reduced condition. Bottom should be smoothened and sloped to facilitate draining of organic waste and toxic substances. Central drainage canal in the pond may also help in the removal of organic waste periodically.

Organic matter:

Unutilized feed, carbonaceous matter, dissolved solids, faecal matter, dead plankton etc. settle at the pond bottom and results in the accumulation of organic

loads. The change in the bottom in terms of increasing organic load should be recorded regularly for the pond management.

Water Quality

Water quality deterioration is related to a decline in pond bottom quality as a result of sediment accumulation. Hundreds of water quality variables may affect aquaculture but, fortunately, only a few normally play a decisive role.

Critical water quality parameters

Water quality variables such as salinity and temperature are important when assessing the suitability of a site for a culture of particular species. Other properties such as alkalinity, turbidity, compounds of phosphorus and nitrogen are important because they affect plant productivity, which in turn, may influence aquaculture production. DO, CO_2, ammonia and other factors come to the play during grow out period, because they are potential stressors for the animal in culture.

Physical characteristics

1. Water temperature

Temperature of water is obviously very important. All metabolic and physiological activities and life processes such as feeding, reproduction, movement and distribution of aquatic organisms are greatly influenced by water temperature. Temperature also affects the speed of chemical changes in soil and water, and the contents of and pressure of dissolved gases. Optimum level of pond water is 25-30^{0}C.

2. Salinity

Salinity refers to the total concentration of all ions in water (Calcium, magnesium, sodium, potassium, bicarbonate, chloride and sulphate). It determines osmotic relationships and also affects the growth, reproduction and migratory behaviour of the fish as well as its general metabolism. As the salinity of water increases, the solubility of DO decreases and the toxic un-ionized ammonia decreases.

Normal level of salinity is around 10-32 ppt. The stress response associated with the sudden decrease in salinity was much reduced when the calcium concentration of the low salinity was increased from 84-150 ppm.

3. pH

The initial pH of pond waters (before biological activity adds to or removes CO_2 to water) is a function of the total alkalinity of the water. pH of most pond water is determined by interactions among dissolved CO_2, carbonic acid, bicarbonate, carbonate and carbonate containing minerals. The proportion of total ammonia existing in the toxic, un-ionized form (NH_3) increases as the pH increases. High pH increases algal bloom formation and reduce swimming performance of fish due to ammonia

accumulation. Low pH increases nitrite toxicity and also the fraction of H_2S (toxic form). Chlorine and metal such as copper, cadmium, zinc and aluminium are affected by pH.

The pH of Brackishwater is usually not a direct threat to the health of the aquatic animal, since it is well buffered against pH changes. Calcium is a particularly important modulator of pH toxicity because calcium affects the permeability and stability of biological membranes. Optimum level of pH is 7.5-8.5.

4. Turbidity

Turbidity refers to an optical property of water that causes light to be scattered or absorbed rather than transmitted through the water in a straight line. Turbidity caused by plankton is desirable whereas turbidity resulting from suspended particles of clay is undesirable in aquaculture ponds. It will restrict light penetration, adversely affecting plant growth and destroy benthic organisms. In case of very high turbidity, fish die due to gill clogging. High value of transparency (>60 cm) is indicative of poor plankton density and the water should be fertilized with right kind of fertilizers. Low value (<20cm) indicates high density of plankton and hence fertilization rate and frequency should be reduced. Optimum range of transparency is 25-35 cm.

Alum (Aluminium sulfate) is an excellent coagulant and is used widely in water-treatment plants to clarify the water. Calcium sulfate, calcium hydroxide calcium ferric chloride, organic matter, certain synthetic polymers and chemical fertilizers are used in removing suspended solids from ponds.

5. Total solids

Organic and inorganic, settleable, suspended (TSS) and dissolved matter is termed as total solids. Portion of organic and inorganic solid that settles in 1 hr in an Imhoff cone is known as settleable solids and dissolved solids are portion of organic and inorganic solids which is not filterable. Portion of inorganic and organic solids that are not dissolved are suspended solids. Optimum level of TSS is < 100 ppm.

Chemical characteristics

1. Dissolved oxygen

The availability of dissolved oxygen frequently limits the activities and growth of aquatic animals. If DO concentrations are consistently low, aquatic animals will not eat or grow well and will be susceptible to infectious disease. If concentrations fall to very low levels, the animals may die.

The concentration of toxic substances such as unionized NH_3, Hydrogen sulphide and carbon metabolites (methane) increases when low DO level exists. However, in the presence of optimum level of oxygen the toxic substances are converted into their oxidized and less harmful forms. Optimum DO concentration for aquatic animal growth is 3-10 ppm.

Use of aerators result in mixing of water at surface and bottom and breakdowns DO stratification and also can eliminate black mud formed at interface of pond water and bottom mud. Water exchange is the best solution to prevent low DO problem in the pond where aeration is not practiced.

2. Total alkalinity

Total alkalinity is the sum of titrable bases in water predominantly bicarbonate and carbonate. Alkalinity of pond water is determined by the quality of the water supply and nature of pond bottom soils. Alkalinity is the capacity of water to buffer against wide swings in pH and enhanced natural fertility of water. Ponds have a total alkalinity of 20-150 ppm have sufficient supply of CO_2 for phytoplankton and it may improve productivity. It also decreases potential of metal toxicity. Very high alkalinity (200-250 ppm) coupled with low hardness (less than 20 ppm) results in rise in afternoon pH beyond 11 and cause death of fish.

Dolomite, Shell lime and Zeolite improve alkalinity and stabilizes pond water quality.

3. Total hardness

Total hardness is the sum of the concentrations of calcium and magnesium in water expressed as mg/l equivalent $CaCO_3$. Nature of water supply largely determines the hardness of ponds.

Total hardness is an indicator of the degree of mineralization of water, and as total hardness increases, concentrations of most other substances tend to increase. Total hardness strongly correlated with alkalinity. Low hardness water contains insufficient calcium ions for protection of fish against acidity and metal toxicity.

4. Carbon Dioxide

Carbon dioxide is a highly water soluble, biologically active gas. It is produced in respiration and consumed in photosynthesis. It is required for plant growth and its availability may limit primary productivity of some aquatic ecosystems. In aquaculture ponds, dissolved CO_2 can be a stressor of aquatic animals and it influences the pH of water.

Dissolved CO_2 concentrations in aquaculture ponds usually range from 0 mg/L in the afternoon to 5-10 mg/L or more at dawn. Application of CaO and $Ca(OH)_2$ can remove excess carbon dioxide.

5. Chlorine

Chlorine used as disinfectant during preparation for stocking, to destroy disease organisms, control phytoplankton abundance and improve water quality in ponds. Free and combined residual chlorine are extremely toxic to fish. The total chlorine residuals

should not exceed 0.002 mg/L as Cl_2 for salmonids and 0.01 mg/L as Cl_2 for other aquatic organisms.

Intense aeration, Addition of 1 mg/lit of sodium thio sulfate for every mg/L of chlorine and exposure to sunlight are some of the management practices.

6. Nutrients

Total nitrogen includes organic nitrogen, ammonia, nitrite and nitrate. Organic nitrogen is bound nitrogen into protein, amino acid and urea. Nitrate is final product of nitrification of ammonia and is a major phytoplankton nutrient. Total phosphorus exists in organic and inorganic form. Organic phosphorus is bound in organic matter and inorganic form of phosphorus exists as orthophosphate and polyphosphate. Nitrogen and phosphorus along with carbon and other trace elements serve as nutrients thus accelerate the growth of phytoplankton, which is the base of the food web in culture system.

7. Primary productivity

Primary productivity is one of the most important sources of energy input in aquatic ecosystem. It is directly related to the temperature and the available nutrients in water and soil. Among the different type of nutrients, nitrogen, phosphorus and potassium are the essential prerequisites for productivity of any aquatic system. Maximum concentration of nitrate-N recorded during monsoon, while minimum during pre-monsoon. Phosphorus regulates the phytoplankton production in the presence of nitrogen.

Toxic metabolites in Brackishwater aquaculture

1. Ammonia

Ammonia is the principal nitrogenous waste product. As ammonia in water increase, ammonia excretion by aquatic organism diminishes, and levels of ammonia in blood and other tissue increases. Total ammonia concentrations are generally highest in ponds receiving large amounts of feed, and at the time of reduction of ammonia assimilation by phytoplankton. Un-ionized ammonia is determined by total ammonia concentration, pH, and water temperature and to a lesser extent on salinity. It is considered more toxic form of ammonia due to its ability to diffuse readily across cell membrane.

Toxic effect of ammonia may be minimized by maintaining sufficient level of dissolved oxygen, Periodic partial removal of algal blooms and water exchange

2. Nitrite

Nitrite is an intermediate product in the bacterial nitrification of ammonia and nitrate. Nitrite is highly toxic to fish as it oxidizes hemoglobin to form methemoglobin, which is incapable of transporting oxygen. Nitrite toxicity is affected by water pH and the presence of Chloride and Calcium ions. Toxicity increases with increasing pH and

decreases with increasing calcium and chloride concentrations. Optimum level of nitrite is less than 0.2 mg/L. Optimum level can be maintained by effective removal of organic waste, adequate aeration and correct application of fertilizer.

3. Hydrogen Sulfide

Hydrogen Sulfide is produced in pond bottom soils under anaerobic conditions and is extremely toxic to aquatic animals. Unionized H_2S concentration is dependent on pH, temperature and salinity and is mainly affected by pH. It regulates the sulfur forms (H_2S, HS & S^{2-}). Un-ionized H_2S is toxic and it decreases rapidly with increasing pH.

H_2S builds up mostly in sediment which is highly reduced (redox potential < 150 mv), within a pH range of 6.5 – 8.5 and low in iron.

Sulfide can be reduced by aeration, water exchange and circulation of water to minimize anaerobic zones in the pond bottom. Application of lime or Potassium permanganate or iron oxide will reduce the hydrogen sulfide. Iron reacts with H_2S and forms insoluble iron sulphide. Periodic pond draining and drying of bottom muds will result in oxidation of sulfide and enhance the decomposition of organic matter.

Safe level of H_2S is < 0.003 ppm.

Conclusion

To ensure sustainable fish production, soil and water quality are two major parameters. Soil quality parameters should be monitored and suitable remedial measures should be undertaken during crop culture and after harvest. Similarly, critical water quality parameters should be maintained within optimum levels by using suitable management practices throughout the culture period. Daily monitoring of the pond conditions and fish behavior along with accurate record keeping helps the farmer to recognize and prevent deleterious environmental conditions in the pond and thereby maximize the production and profit.