TRAINING MANUAL
ON
BRACKISHWATER AQUACULTURE

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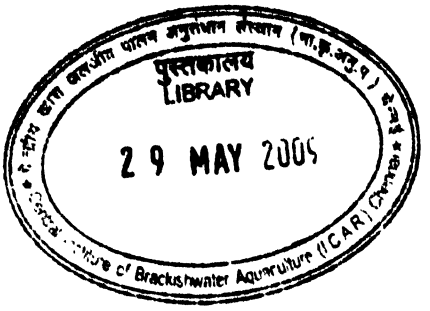

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CIBA, ITS MANDATE AND ROLE IN BRACKISHWATER AQUACULTURE DEVELOPMENT IN INDIA

India has a long coast line of 8129 km distributed in nine coastal states and four Union Territories. The biodiversity of the coastal ecosystem of the country is rich with a wide spectrum of fauna and flora. The country is bestowed with 3.9 million ha estuaries, 1.2 million ha brackishwater area, 2.54 million ha salt affected coastal soils and 8.0 million ha inland saline soils. These resources are the real biological wealth and strength and provide immense opportunities for development of brackishwater aquaculture in the country.

India is the third largest producer of fish in the world and ranks second in world inland fish production. In shrimp aquaculture, the country occupies fourth position. The fish production in the country has shown remarkable growth with 6.3 million tones in the year 2004-05. The fisheries sector contributes around Rs. 29,707 crores to the country's economy, 1.04 % of the national Gross Domestic Product (GDP) and 5.34 % to the agriculture GDP. About 56% of the population consumes fish and the per capita consumption of fish is 9 kg/year against the global per capita consumption of 12 kg/year. The brackishwater aquaculture sector has great potentials to meet the challenges of the food security basket of the country and to generate employment and attract foreign exchange. Brackishwater aquaculture is synonymous to shrimp aquaculture and it is centered on one species, *Penaeus monodon*. Shrimp aquaculture has shown rapid growth in the last decade and the production increased from 30,000 t in 1990 to 1,43,170 t in 2005-06 and this has resulted in the all round development of shrimp hatcheries, feed mills, ancillary industries like ice plants, processing plants, drugs, chemicals and other aquaculture related engineering products.

Realizing the need for a research thrust in brackishwater aquaculture, the Indian Council of Agricultural Research (ICAR) established Central Institute of Brackishwater Aquaculture (CIBA) on 1st April, 1987. The Institute's main office is situated at Chennai and only regional centre at Kakdwip, West Bengal. The Institute has established state of art laboratories for research in aquatic animal health, nutrition, biotechnology, environmental biology etc. Over the years the Institute has generated a wealth of information on shrimp, fish and crab production systems, nutrition and feed technology, farm environment, genetics and biotechnology, breeding and seed production of candidate species, disease diagnostics, aquaculture engineering, farm and hatchery designs, database on the various brackishwater aquaculture systems practiced in the country and the extension practices.

CIBA was mandated to develop techno-economically viable and sustainable culture system for brackishwater finfishes and shellfishes and to transfer the technologies for the benefits of the different stakeholders of the sector. CIBA has made significant research achievements and these include development of cost effective technology packages for induced maturation of *P.monodon*, backyard hatchery technology for *Fenneropenaeus indicus*, culture and cyst production of Artemia, domestication of *Marsupenaeus japonicus* and production of F5 generation, improved traditional culture of P. monodon with 1.5-2.3 t/ha production, pond culture of *M. japonicus* with one ton production, development of shrimp feeds with FCR comparable to commercial feeds, micro particulate feed for seabass larvae, PCR diagnostic kits for WSSV, WMD and MBV, shrimp immunostimulants, package for estimation of carrying capacity of source water body, seabass hatchery technology, captive seed production of pearlspot, polyculture of milkfish and shrimps, integrated shrimp/fish culture with poultry, breeding and seed production of mud crabs and grow-out culture of seabass. These research outputs have contributed to the growth of the sector and provided a platform for interaction with the farmers and industry for further advancements.

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PRESENT STATUS OF BRACKISHWATER AQUACULTURE

T. K. Ghoshal

Aquaculture is the fastest growing food production sector accounting for about 50% of food fish production, with average annual growth of 8.8% during 1950-2004. FAO in 1998 (FAO 1998) predicted that world aquaculture production will reach between 35 to 40 million tons of finfish, crustaceans and mollusks by 2010. However, the global production reached 59.4 million tons by 2004 (including aquatic plants) from less than a million tons in 1950, with farm gate value of over $ 70 billion, surpassing the predictions and it is further expected to increase. Of this production, 69.6% or 41.3 million tons was produced in China alone. India with 2.5 million tons stands second in world aquaculture production. It is expected that by 2025, globally one out of every two fish consumed will be from aquaculture. In spite of this, aquaculture lags behind agriculture in application of science and technology and in value chain productivity. Indian aquaculture has shown an annual growth of 8%.

In the past, aquaculture was an activity mostly of Asian countries and was mostly focused on freshwater aquaculture that too cyprinid based, but now it has spread to all continents, all aquatic environments and many species. Ninety percent of global aquaculture production comes from Asia and of this nearly 70% is contributed by China. The ten major aquaculture-producing countries are: China, India, Vietnam, Thailand, Indonesia, Bangladesh, Japan, Chile, Norway and United States of America. Asia and Pacific region produces 99.8% of cultured aquatic plants, 97.5% cyprinids, 87.4% of penaeids and 93.4% of oysters. India with a production of 2.5 million tons (4.2% of global production) is the second largest aquaculture producer. However, when we compare with the production from China, which amounted to over 30 million tons, India's production appears little. With vast aquatic resources, India has high potential for increasing production from aquaculture.

In spite of technological advances in terms of improved breeding and seed production technologies, improved fish nutrition and better control of disease, aquaculture is still an infant industry with vast potential for growth Nearly 80% of aquaculture production comes from small-holder operated farms. Thus, small-holder farming families are not only consumers of fish, but also producers. If we are to

benefit this community and at the same time meet the growing demand, we have to address the issues of access to resources - both natural and financial, lack of skills, vulnerability and aversion to risks. This would mean understanding contextual circumstances, operating environments and the conditions that enable the poor to take advantage of opportunities. At the same time we have to develop opportunities for enterprise development. With land and water becoming scarce we cannot afford at concentrating our efforts on extensive aquaculture. We have to intensify the culture systems without impacting on the environment.

Brackishwater aquaculture

Brackishwater aquaculture presently is synonymous with coastal aquaculture and mainly dependent on a single species, tiger shrimp, *Penaeus monodon*. There is a high potential for species diversification with other shrimps and crabs.The other potential shrimp species like *Fenneropenaeus indicus, Fenneropenaeus merguiensis, Penaeus pencillatus, Marsupenaeus japonicus* and *Peneaus semisulcatus* are not cultured on a commercial level larges scale culture. Mud crab species, *Scylla tranquebarica* and *S. serrata* are ideal aquaculture candidate species. The fin fish species like the seabass (*Lates calcarifer*), grouper (*Epinephelus spp.*), grey mullet (*Mugil cephalus*), peral-spot (*Etroplus suratensis*), milk fish (*Chanos chanos*) which are promising and ideal for aquaculture has not been exploited. The fishes like snapper, threadfin bream, rabbit fish, silver pomfret, seabream, spotted scat, moon fish can be cultured in coastal area. Recently with the development of seed production technology of seabass by CIBA, and setting up hatcheries for large scale production of seabass seed by CIBA and Rajiv Gandhi Centre for Aquaculture (RGCA), the aquaculture of seabass is getting momentum in India. However with increasing salinisation of inland soils, spreading to over eight million hectares, inland saline aquaculture could become an important economic activity in the years to come. The major problems of the sector, *viz.*, lack of disease-free shrimp seed, slumping prices of shrimp in overseas markets and lack of diversification are impacting on the growth. While efforts are being made to produce specific pathogen-free shrimp seed both through selection programmes in the country and establishment of a multiplication centre for SPF seed with Hawaiian technology, domestic markets are being pursued and diversification protocols for *Penaeus vennament*, seabass farming and crab fattening formulated.

Ensuring pathogen-free broodstock being the major challenge, it is necessary that appropriate quarantine and biosecurity measures are adopted in the hatcheries as an immediate measure. In order to overcome the problem of deficiency of broodstock, development of captive broodstock and domestication can be an alternative for supply of disease-free seed.

Development of environment-friendly and cost-effective culture technologies of both shrimp and finfish focusing on small-scale farmers is in the need of the hour. Protocols for better management of soil and water resulting in reduction of pollution can substantially reduce the risk. The risks of diseases like loose shell syndrome in grow-out culture system can Monodon baculovirus in hatcheries need to be tackled. Further, development of diagnostic techniques for other exotic viruses like yellow head virus and Taura Syndrome virus are to be given utmost attention in view of their possible threat in the coming years. A comprehensive health management approach in shrimp farming including development of effective therapeutics, probiotics and vaccine is to be formulated.

Since, brackishwater aquaculture is presently mainly dependent on exports, issues such as traceability and anti-dumping are expected to have a significant influence on growth of the sector. Development of a strong domestic market for the produce, establishing a well-knit system for market information and intelligence for aquaculture production are other aspects needing trust. Implementation of aspects HACCP, traceability, eco-labeling and quality assurance criteria for uniform and wider compliance is necessary for building the confidence of the importing nations and boosting the export of our produce.

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BIOLOGY OF CULTIVABLE BRACKISH WATER SHELL FISHES

P.S. Shyne Anand and A.Panigrahi

Introduction

Cultivable brackish water shell fishes include penaeid shrimps and brachyuran mud crabs. According to FAO, there are over 300 shrimp species are available throughout the world. However, only few species are commercially important either in capture fisheries or in the aquaculture industry. The main cultured penaeid shrimps across the world are the giant tiger shrimp (*Penaeus monodon*), the fleshy prawn (*P.chinensis*), the white leg shrimp (*P.vannamei*), the Indian white shrimp (*P.indicus*), the banana shrimp (*P.merguiensis.*) and kuruma shrimp(*P. japonicus*). Among the 700 marine littoral crabs of India, only two species of mud crabs *Scylla tranqubarica* and *S. serrata* are commercially cultured in brackish water ponds. Cultured shrimps and live mud crabs are continues to be the main shell fish commodity in export market.

Biology of commercially important Penaeid shrimps

Distribution: Penaeid shrimps are widely distributed in Indo-west Pacific water bodies. They are mainly cultured in coastal and off shore waters of both eastern and western hemisphere.

Habitat: Adults penaeid shrimps generally found in off shore waters and spwan in the salinity regime of 30-35 part per thousand (ppt) at a depth of 30-100m. Juveniles often prefer brackish waters of estuaries and coastal wetlands, while larval stages inhabit plankton-rich surface waters off-shore, with an on-shore migration as growth advances.

Morphology: The rostrunm extends beyond the tip of the antennular peduncles and has generally 6-8 dorsal and 2-4 ventral teeth. In penaeids, adrostral carina reaches almost to the tip of epigastric tooth and carina reaqched to the posteriar edge of the carapace. The abdomen is carinated dorsally from the anterior one third of the 4th to 6 th somites and the telson is unarmed.

Life Cycle: The penaeid life cycle includes several distinct stages and they are generally found in a variety of habitats. Adults migrates to the sea for breeding. During spawning, eggs and sperm are simultaneously released from the female. Fertilization is external, and egg development occurs in the water column. The fertilized eggs are demersal and hatch within 14 hours to strongly phototropic nauplii (6 sub stages, each moult every 4-6 hr interval), which swim towards the surface. After 36 hr, the larvae pass through distinct stages, protozoea (3

sub stages, each moult 1 day interval) and mysis (3 sub stage, each moult 1 day interval), before metamorphosing into postlarval shrimp. Larvae are are planktonic.The early post larvae become benthic and are adapted to tolerate wide range of salinity fluctuation. So, PL and juveniles migrate to brackish waters of estuaries and coastal water bodies.

Food and feeding habits: Penaeid shrimp are known to ingest a variety of items and have been described as omnivorous, detritus feeders and carnivores. Their diet ranges from the hereditary yolk sack, during the early naupliar stage to phytoplankton at protozoeal stage and then to zooplankton at mysis stage. Epibenthic postlarvae and juveniles consume both animal and plant matter, including microalgae, detrital aggregates, macrophytes, foraminiferans, nematodes, copepods, tanaids, larval molluscs, and brachyuran larvae. As shrimp grow, they consume mysid and caridean shrimp, amphipods, polychaetes, and molluscs, as well as fishes. Sub adult and adult shrimp also consume significant amounts of detrital aggregates.

Moulting: Shrimp increases in size through a physiological process called moulting cycles. Moulting begins with an increase in concentration of molting hormone in the hemolymph. During moulting, a shrimp undergo continuous process like periodically loosen the connectives between their epidermis and the extracellular cuticle; rapidly escape from the confines of this rigid cuticle; take up water to expand the new, flexible exoskeleton; and then quickly harden it with minerals and proteins. Ecdysis, as a stage, only lasts a few minutes. It begins with the old exoskeleton opening at the dorsal junction of the thorax and abdomen in decapod crustaceans, and is completed when the animal escapes from its confines. Different stages of the moulting process includes premolt (proecdysis), moulting, postmolting and intermoult.

Digestive system: The morphology of the digestive tract in the penaeid shrimp is similar to that of most Decapoda. It is divided into a complex, cuticle-lined foregut region (proventriculus); a compact digestive gland, hepatopancreaes at the beginning of the midgut region, followed by a long tubular, mid gut gland and a cuticle-lined hindgut region. The mouth leads into a short vertical oesophagus, which opens into the lumen of the anterior of the forgut. The proventriculus is divided into two principal chambers. The anterior chamber is distensible, called the cardiac stomach, and has a pair of ventro-lateral plates, gastric mill and a dorsal median tooth. The posterior chamber, pyloric stomach is much narrower which open into to the midgut, through filter-press. The principal functions of the midgut are the secretion of digestive enzymes and absorption of nutrients. The remainder of the midgut is a straight tube, running from the cephalothorax dorsally through the abdomen to the rectum. The short muscular rectum is lined by six pad-like ridges, whose primary function appears to be for grasping the faecal pellet in the peritrophic membrane and extruding it.

Reproductive system: Penaeid shrimps are sexually dimorphic with distinct external features. The male has two pairs of modified abdominal appendages on the first and second abdominal segments namely the petasma and appendix masculine respectively which are modified for spermatophore transfer to the female's external receptacle, the thelycum which is located between the bases of the fifth walking legs. The thelycum may be "open" or "closed", depending on the species. In closed thelyca species, thelycum is enclosed by chitious plates and spermatophore is placed inside the grove where as open thelyca are not enclosed by plates, and the spermatophore must be placed on it by a male when the female's exoskeleton is hard. Usually females have open thelycum spawn immediately after mating unlike closed thelycum species where there is a time lag between mating and spawning. The open thelyca are found in some shrimp species endemic to the Western Hemisphere, such as *P. stylirostris* and *P. vannamei*; while closed thelyca are characteristic of most Asian species, such as *P. monodon*, *P. chinensis*, *P. indicus* and *P. merguiensis*.

Internal organs of the reproductive system: Male reproductive system includes a paired testes, vas deferens and terminal ampoules for spermatophore storage. The female reproductive system includes paired (but partially fused) ovaries that extend from the mid-thorax to the posterior end of the abdomen, and oviducts terminating adjacent to a single thelycum.

Biology of commercially important mud crabs: Commercially important mud crabs belong to the family portunidae. The main cultivable brackish water mud crabs are *Scylla tranquebarica* and *S.serrata*. Mud crabs are generally sold in live condition both in domestic and export market. Live and gravid female mud crabs above 300g size is much sought after commodity in export market.

Distribution: The mud crabs are economically and recreationally important brachyuran crabs distributed in the shallow coastal waters, brackish water lakes, and lagoons and inter tidal mangrove areas of the Indo-West Pacific region.

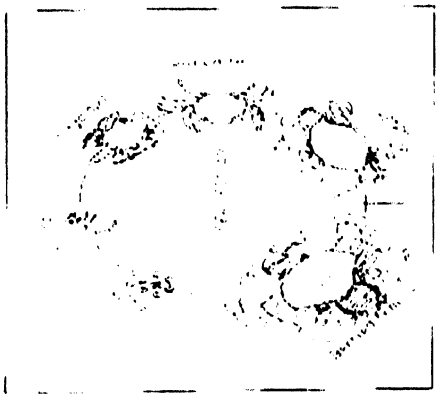
Habitat: Adult mud crabs are generally found in muddy, mangrove-lined estuaries, and the ovigerous females move off shore to spawn. Crabs which have a dispersive coastal larval stage and occur within estuaries as adults usually colonize coastal habitats as megalopae or postlarvae. *S.tranquebarica* is free living and frequently seen in open estuaries where as *S.serrata* is burrowing in nature. They are mainly euryhaline in nature and able to tolerate 0-45 ppt salinity level. However, in grow out condition they grow well in 10-30 ppt salinity.

Sexual identification: Mud crabs are sexually dimorphic and can be distinguished once it reaches 35 mm carapace width (CW). Abdominal flaps are slender and triangular in case of males where as it is triangular and broad in female. In fully matured and berried female abdominal flap is semi circular or half moon in shape.

Food and feeding habits: Mud crabs are generally carnivorous in nature. They mainly feed on bottom dwelling animals, small crustaceans and decayed animal matters. In culture condition they accept formulated feeds.

Moulting: The moulting process depends on body size, physiological and environmental factors. During moulting animal uptake water and minerals get absorbed from the exoskeleton and then it is cast off. After moluting tissue is get replaced with water. The newly moulted crab will be having more water in the tissue and generally know as water crabs or soft crabs. Frequency of moulting decreases as animal age increase. Larger species generally attains a size of 210 mm CW /2.4 kg where as smaller ones attains a maximum size of 700kg/140 mm CW.

Sexual maturity and breeding: Usually, the mud crab reaches the reproductive stage when its shell width is greater than 7.8 cm and its body weight over 100 g. Breeding season varies all along the coast. During mating female store spermatophore in its body and when egg become ripe they get fertilized and attach to the pleura .Mud crabs are continuous breeders and berried females occurs throughout the coastal waters. However peak breeding season varies place to place. Fecundity vary from 2-3 million eggs in larger species and 0.2-0.3 million in smaller ones. Incubation period is 2 weeks and during that time colour of the berry changes from orange to brown, then to black.



Larval life cycle: There are 5 zoeal stage which moult within 2-3 days interval and one megalopa stage which takes 11-12 days to reach first crab instar stage. Zoeal stage onwards it starts its cannibalistic nature. The megalopa larvae gradually adapt themselves to a benthic life. Because of their phototaxic behaviour, larvae are often attracted by light at night.

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BIOLOGY OF CULTIVABLE BRACKISHWATER FINFISHES

G. Biswas

Several species are available in brackishwater environment, but commercial farming is restricted to only few species due to some important factors, viz., economic importance, growth rate, culture compatibility, seed availability etc. It is useful to have knowledge on basic biology of any animal before undertaking their handling and maintenance. Here, biology of some brackishwater species having economic importance is discussed.

1. Biology of Lates calcarifer

The Asian seabass, *L. calcarifer* is a much esteemed food fish and it belongs to the family Centropomidae.

1.1 Food and feeding habits

Seabass is carnivorous in nature. However, juveniles are omnivores. It is opportunistic predator and its diet changes with size. Stomach contents of smaller fish (1-10 cm) showed 20% phytoplankton and 80% fish, shrimp etc. In bigger fish (20 cm), the gut contained 100% animal prey (70% crustaceans and 30% fishes). It prefers pelagic fishes than benthic crustaceans. It also has cannibalistic habit.

1.2 Size at maturity

Seabass is a protandrous hermaphrodite fish. Majority of individuals from early age groups are males weighing 2.0-3.5 kg body weight, but after attaining 4 kg and above (4 years old), the majority of them become females. Males attain maturity at 25 cm in total length and females mature at the size of 65-85 cm.

1.3 Maturation

Gonadal development is very rapid just before spawning and coinciding with fast growth. The gonads are strongly dimorphic and the gonad size varies in different growth stage. Usually, oocytes in the posterior end of ovary are larger in size than the oocytes of anterior region indicating the process of continuous ovarian development and occurrence of multiple spawning. In fully mature females, the diameter of oocyte usually ranges from 0.45-0.53 mm.

1.4 Spawning season

Seabass spawns during April to November in Indian waters. Spawning takes place in sea. It is a multiple spawner and releases eggs in batches continuously upto 3 days. Fertilized eggs are usually transparent, pelagic and easily drifted by tides towards coastal areas for larval development. Restoration of gonads takes place during onset of north east monsoon in the Indian east coast.

1.5 Sex ratio

In induced breeding operation sex ratio of 2:1 (male to female) is maintained for proper fertilization.

1.6 Fecundity

Fecundity of seabass depends on the size and weight of fish. It varies between 1.0 to 20.0 million eggs.

1.7 Age and growth

Normally fish attains 0.8-1.0 kg in the first year and 2.0 kg in the second year. Sometimes large size seabass even upto 6.0 kg is caught from estuaries and inshore waters using hook and line. It shows wide growth variations under culture condition. Even though same size group seed stocked together, due to natural differential growth in the first year itself, fishes are available from 0.4 to 4.0 kg indicating its growth potential.

2. Biology of *Chanos chanos*

Milkfish, *C. chanos* is the only member of the family Chanidae. It is an important fish from aquaculture view point and cultured in large scale in South East Asia.

2.1 Food and feeding habits

Milkfish is an herbivorous fish. But their larvae feed mainly on zooplankton. Juveniles and adults eat cyanobacteria, soft algae (Cyanophyta, *Lyngbya spp.* and diatoms) small benthic invertebrates, decayed organic matter and even pelagic fish eggs and larvae. The plant animal complex, namely *lab-lab* formed in shallow water is one of the preferred food items. It has fine gill rakers and long intestinal tract which help in retaining and digesting this kind of food ingested. They can be adapted to accept artificial diet very easily.

2.2 Size at maturity

They attain maturity at an age of 5-7 years with body weight of 3 kg and above.

2.4 Spawning season

In Indian peninsular coast, milkfish spawns during December to May. Seed collection centres are on east coast, Rameswaram, Mandapam and Pulikat Lake in Tamil Nadu and Cochin coast in Kearal on the west coast.

2.5 Spawning

Spawning takes place in offshore waters, 15-30 km away from the shore at a depth of 20-30 metre in clear water over sandy or coral bed.

2.6 Fecundity

Induced maturation and spawning have been successfully done in the Philippines, Taiwan, Hawai and Indonesia. The fecundity is estimated as 2 million eggs/ kg body weight.

2.7 Habitat and growth

It is a euryhaline species which can withstand sudden changes in salinity and can be grown in fresh, brackish and marine waters. Its salinity tolerance limit is 0 to 158 ppt. It can tolerate a temperature range of 15-40^{0}C, but the optimum is between 20^{0}C and 33^{0}C. Milkfish is a marine species with catadromous migratory habit. Adults spend part of their lives in littoral waters and go to sea for breeding. In nature it grows to a maximum of 1.5 m. In well maintained culture ponds it grows to a marketable size of 300-400 g in 3-4 months.

3. Biology of *Mugil cephalus*

The striped or jumping grey mullet, *M. cephalus* is the most important species of the grey mullets which belong to the family Mugilidae. *M. cephalus* is the fastest growing grey mullet among 77 species and enjoys a very cosmopolitan worldwide distribution.

3.1 Food and feeding habits

Fry of less than 30 mm feed principally on zooplankton. The juveniles feed preferably on diatoms and epiphytic cyanophyceae. Gut contents of adult consisted of sand, decayed organic matter, diatoms, dinoflagellates, foraminifera, algae and miscellaneous items like copepods and tintinnids. Qualitative composition of gut contents says that it is an iliophagous subsisting mainly on decayed organic matter. They feed in a head down position, moving its head from side to side. The movement sometimes is so vigorous that the whole body shakes, as a result a cloud of mud along with soft flocculant matters rich in microorganisms are sucked through its protrusible mouth. Although it can adapt itself to artificial diet, it has a preference for natural food.

3.2 Size at maturity

Age at maturity varies from first year to eighth year in both sexes, and size at maturity in males varies from 230 to 400 mm and in females from 240 to 415 mm.

3.3 Maturation

Ova of different maturity stages are found, but only one distinct group of mature ova with a wide range of size indicates that the fish has a single spawning. The eggs with 0.6 mm diameter are fully ripened.

3.4 Spawning season

The spawning season in India is from October to May and the fry availability along the coastal regions is very seasonal. In Kerala, fry availability is from June to August in the Puduvypu region. In Pulicat Lake, seeds are available during January to March. In West Bengal coast, grey mullet seeds availability is found from February to April.

3.5 Spawning

Spawning occurs in offshore waters where warm water current exists with surface water temperature of 20-23^{0}C during spawning season.

3.6 Sex ratio

Sex ratio in Pulicat Lake water is found 1.56:1.00 fro male to female.

3.7 Fecundity

The estimated fecundity of Pulicat Lake *M. Cephalus* female ranged from 4.34 to 47.17 lakh eggs per female.

3.8 Migration

M. cephalus undergoes 3 types of migrations in its life history. A) Osmoregulatory migration- a phenomenon of juveniles anadromously migrating towards estuaries, B) Seaweed migration- adult mullets migrating towards open sea after being in estuaries for gonadal maturity, C) Spawning migration- the ripe mullets migrating in schools from feeding grounds to spawning grounds in a particular direction.

3.9 Age, growth and habitat

The maximum size reported is 1.2 m and the most common marketable size is 500-800 g (30-50 cm). The rate of growth is highly variable depending on the climatic and environmental conditions. When cultured with Indian major carps, they can grow upto 40 cm in a year. It is a eurythermal and euryhaline species. The maximum temperature tolerance limit is 40^0C. It can be cultured in waters with salinities ranging from 0 to 145 ppt.

4. Biology of *Etroplus suratensis*

The pearlspot, *E. suratensis* belonging to the family Cichlidae is also referred to as green chromid and has high market value in the southern states of India, especially Kerala.

4.1 Food and feeding habits

Young ones feed almost on zooplankton, but from juvenile stage onwards, they are mainly herbivorous and detritivorous and feed on algal weeds, filamentous algae, detritus etc. However, miscellaneous food items such as insects, molluscs, crustaceans and sponges also form part of its food.

4.2 Size at maturity

The fish attains maturity at 8-9 months of age and the size at maturity varies from 10.5-18.0 cm.

4.3 Maturation

There are different maturity stages of gonads, viz. immature, maturing, ripening, ripe and spent. At ripe stage ovaries measure 30-52 mm in length with largest group of ova of 2 mm diameter. Whereas, for male, testes measure 32-48 mm in length and with little pressure, milt oozes out.

4.4 Spawning season

It breeds throughout the year but two peaks have been noticed, one from December to February and the other from July to August.

4.5 Breeding behaviour

The breeding behaviour of pearlspot is complex involving courtship, pairing, nest-building and parental care. Before pair formation they move in group and courtship starts between some members of the groups and pairs are formed. Nest-building involves selection of nesting materials and preparation of the nest for laying the eggs. After spawning, fertilized eggs are placed in the nest and there may be 1000-6000 eggs in a nest. Parental care involves care of the eggs and the young ones. After 4-5 days of spawning eggs hatch out. The newly hatched larvae are picked up by mother into her mouth and transferred to pits measuring 6-8 cm diameter with 2-3 cm depth. These pits are made ready before the eggs hatch out. Parents actively produce a constant current near pits by fanning with fins. After yolk sac absorption, the larvae are led out as the pectoral fins become functional. Parental care lasts for a considerable time even after the young ones assume adult forms (upto 40 to 50 mm).

4.6 Sex ratio

Females dominate over males in natural waters. The sex ratio in different size groups was reported as 1:0.84 to 2.73:1 (female:male) from Indian coast.

4.7 Fecundity

In general, the fecundity of perlspot is low ranging from 500 to 6000. But it also depends on various parameters such as fish size, ovary size.

4.8 Age and growth

Males are bigger than females and exhibit better growth rate (150-175 mm/ 125-150 g in a year). It can grow over 30 cm in length and 1.35 kg in weight under favourable conditions. In Chilka lake areas they grow upto 105 mm in the first year.

5. Biology of *Scatophagus argus*

The spotted scat, *S. argus* is a euryhaline teleost widely distributed in nearshore waters of Indo-Pacific region. It is popular aquarium fish and an important food fish in its available areas.

5.1 Food and feeding habit

The binomial nomenclature *Scatophagus argus* is translated from Greek as 'spotted faeces-eater', and was derived from the habit of scat to gather in harbours and feed on offal and other wastes dumped from ships. It is uncertain, however, if scats are true coprophages since their acceptance and/or preference for faecal matter has not been confirmed. Gut content analysis revealed that adult scats are primarily herbivorous. They accept green filamentous algae and brown seaweeds also. Worms, crustaceans and insects constitute part

of their food items. The evidence of herbivorous food habit is supported by presence of their long coiled intestine approximately 3.5 times the body length.

5.2 Sex determination

Sexes can be differentiated by head shape. In females, head profile ascends at a constant slope, whereas males have a concave curvature of the head above the eye. This difference is more prominent in larger fish of 100 g and above. In addition, females are often a lighter olive green colour compared to darker males.

5.3 Size at first maturity

Size at first maturity varies with sexes. Females with 14 cm (150 g) and males with 11.5 cm (83.5 g) standard lengths show first sexual maturity.

5.4 Spawning season

In West Bengal coast the fishes spawn from June to August during prevalence of south west monsoon wind.

5.5 Spawning

Spawning occurs in water with salinity of 25 ppt or more in river mouth or estuarine areas. Size of spawned eggs are of 0.68-0.75 mm diameter. The eggs are transparent and spherical containing a single oil droplet of 0.30 mm diameter.

5.6 Tholichthys larvae

Scat larvae pass through a developmental stage known as tholichthys. This stage is a unique feature of few genera of teleost, including butterfly fish (Chaetodontidae) and scats (Scatophagidae). These larvae are deep bodied and laterally compressed. They are usually very dark, have rough and scaleless skin and a well developed lateral line. Their size ranges from 0.6 to 1.2 cm. The most distinctive feature of these larvae is bony plate which completely encases the head in a thick protective sheath. One of these plates dorsal to the eye has posteriorly oriented projections which form spiny horns on either side of the head. These plates are slowly absorbed as the tholichthys larvae metamorphose into juvenile forms.

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Biology, fishery, culture and seed production of the pearlspot *Etroplus suratensis* (Bloch). CIBA Bulletin No.7, 1995

Biology and Fishery of Important Grey Mullets of Pulicat Lake. CIBA Bulletin No.11, 1998.

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SITE SELECTION, DESIGN AND CONSTRUCTION OF BRACKISH WATER PONDS

P.S. Shyne Anand, G.Biswas and A. Panigrahi

Introduction

India is bestowed with 1.2 million ha potential area for development of brackish water aquaculture and only 15-16% of the area alone has so far been brought under culture. With good number of candidate species like shrimps, crabs and fin fishes available in the country, there is a vast scope for development of brackish water aquaculture. However, Level of intensification and lack of awareness about the management practices has attributed to the disease outbreak and severe economic losses to the brackish water aquaculture industry. Moreover, improper site selection, lack of good layout plan and design results in various environmental issues like salinisation of agricultural lands and drinking water, destruction and conversion of ecologically sensitive mangrove areas etc. Hence, besides technological aspects of the culture, the environmental and socio economical aspects to be considered before finalizing the site for brackish water farms.

1. Site selection: Selection of a suitable site is the first and foremost step in the design and construction of an aqua farm. A mistake made during the phase of site selection may result in higher cost of construction and culture operation, and create environmental problems as well. A suitable site is one that provides optimum conditions for the growth of species cultured at the targeted production level, given an effective pond design and support facilities. Proper guidelines are to be followed for integrating coastal aquaculture in to the local environment and social settings.

1.1 Location of the sites: The following aspects should be considered while deciding a suitable site for brackish water farms.

- Mangroves, agricultural lands, salt pans, and other ecologically sensitive areas like sanctuaries; marine parks should not be used for shrimp farming.
- Shrimp farms should be located minimum 100 m away from a village with < 500 population, 300 m away from village with > 500 people, 2 km from towns, heritage areas
- All shrimp farms should maintain 100 m away from drinking water source
- The shrimp farms should not be located across natural drainage canals/ flood drain

- Leave enough space between farms for free access to the traditional users the water front

2. Topography: Topography refers to changes in the surface elevation of natural ground i.e. whether the ground is *flat*, sloping, undulating or hilly. The best area for brackish water ponds is where the ground is levelled (flat) or there is a slight slope. Preference should be given for gravity flow of water to facilitate easy pond bottom drying and proper water exchange.Excessive undulating topography should be avoided as it increases cost of construction.

2.1. Site elevation: Elevation of the site from the lowest low water level of the supply creek should be considered while selecting the site. A minimum elevation of 0.4-0.6 m is essential to ensure proper water exchange and drainage by gravity.

2.2. Tidal amplitude: Average tidal amplitude of 1.5-2.0 m is ideal for brackish water farms. Sites having tidal fluctuation > 4 m and below < 1 m should be avoided as it can cause difficulty in water filling & drainage.

3. **Soil type & Quality:** Soil is one of the most important components of a brackish water culture system. Soil quality should be ascertained for pH, permeability, bearing capacity, nutrient status and heavy metal content. Permeability or water retention capacity of soil depends on the soil texture. Clayey loam soil is ideal for brackish water farms as it has low permeability & high fertility. Clayey loam contains textural components like sand: 20-45%, silt: 15-23% & clay: 27- 40%. Area contain sandy soil should be avoided as it causes seepage and salinisation problems. Soil with pH below 5 and high concentration of heavy metals should be avoided.

3.1 Soil characteristics suitable for a brackish water shrimp pond

Soil quality parameters	pH	Organic carbon (%)	Available Nitrogen (mg/100g soil)	Available Phosphorous (mg/100g soil)	Electrical conductivity (mmhos/cm)	Calcium carbonate (%)
Ideal range	7-8	1.5-2.5	50-75	4-6	>4	>5

4. Water Source & Quality of Water: Good quality and adequate amount of brackish water should be available throughout the culture period. The water source could be from brackish water creeks/canal, lagoons or backwaters. The quality of the water available in the site has a strong influence on the success of the shrimp farm. Water quality parameters like pH,

salinity, and dissolved oxygen and the presence of heavy metal should be ascertained. The water source should be free from any industrial or agricultural pollution. Wide fluctuation in salinity and pH is detrimental to the animals.

4.1. Optimum level of intake water quality parameters

Water quality parameters	Ideal range
Temperature(^{0}c)	28-33
pH	7.5-8.5
Salinity(ppm)	15-25
Dissolved oxygen (ppm)	>5
Transparency(cm)	25-45
Mercury (ppm)	<0.001
Cadmium (ppm)	<0.01
Chromium, Copper, Zinc (ppm)	<0.1

5. Infrastructure facility and Hydro meteorological parameters: Apart from the above mentioned factors, other environmental, socio-economic factors and infrastructure facilities to be considered while site selection as these factors play an important role in sustainability and economics of culture duration. Hydro meteorological parameters like prevailing condition of wind and wave action, rain fall, humidity, temperature, frequency of occurrence natural calamities like cyclones, flood etc. to be recorded before construction of farms. Brackish water farms should be accessible with good transportation & marketing facilities. Availability of quality seed, freshwater & power supply should be considered before selecting the site.

6. Pond design and Construction: Proper design and construction of farms are essential for the efficient management of culture ponds. It demands proper planning, careful supervision & skilled workmanship. Proper pond design helps to avoid problems related to water intake and discharge, seepage and erosion, floods, storms etc. A Site specific approach to be followed as site characteristics varies from place to place. Ideal farms are a complex establishment of different size of ponds for nursery and grow out. 10-15% of the area can be used for nursery ponds and 65-70% area for grow out ponds. However, place allotment varies for species to species. Construction procedure includes land clearing and marking, excavation of ponds, construction of dykes and sluice, water supply canal, laboratory and watchman sheds.

6.1. Design & Construction of Dike: Embankments are designed to prevent flooding and erosions apart from serving as boundaries to indicate pond size and shape. Two types of dike are generally constructed in brackish water ponds.

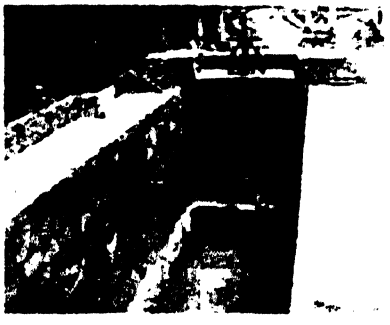
6.1.1. Periphery dike (Main dyke):- Periphery dike protects the entire farm against flood and tidal action. The dyke is constructed based on the on the highest high tide level, water currents, wind action, expected vehicle load etc. Compactability of the dyke is increased by using impervious materials (concrete or clay bags) as the core of the dyke. A slope of 1:1 (H: V) for clay soil and 3:1 for sandy soil is recommended based on the type of soil.

6.1.2 Internal dike (Secondary dike): Internal dike are constructed to partition between ponds. The height of the dike depends on the designed pond water level. Top width range from 1.2m to 2 m

6.1.3 Free Board: It is the vertical distance between crest & water level. A free board of minimum 0.6 m and 0.3 m above desired water depth should be for periphery dike and secondary dike respectively. Proper shrinkage allowance 10-20% to be given based on the soil type.

7. Water Intake & Supply System: Design of water intake and supply canal depends on the daily water requirement. Depending on the soil quality, earthen, stone pitched or concrete canal can be designed. PVC pipes can be used for the water supply system.

7.1 Water control gates (sluice gates): While designing sluice gate it is essential to consider tidal fluctuation in order to ensure effective control of water flow. Sluice gates are classified in to main sluice gate and secondary gates. Main sluice gates are situated at the periphery dike and secondary gates in the individual ponds. Wooden shutters are used to regulate the entry and exit of water flow in to the ponds. The coarse and fine meshed screens are used in the outlet sluice gate to prevent the entry of unwanted organisms. Separate Inlets and outlets should be constructed and must be diagonally placed for proper drainage.



Main sluice gate

Secondary sluice gates

8. Pond Shape, Size & Depth: Rectangular are ideal for grow out ponds with its long axis remain parallel to the prevailing wind direction for proper aeration. The size of the pond good for management is between 1 to 2 ha. A minimum depth of 0.8 m to 1.2 m recommended for shrimp culture and 1.5-1.8 m is ideal for fish ponds. As per Coastal Aquaculture Authority, 60% of total farm area should be water spread, rest 40% for other purposes.

9. Effluent treatment ponds: According to CAA rules, a minimum 10% of water area should be used for Bio-ponds if farm size is > 5 ha farm. It is also necessary that smaller farms that are located in close proximity to each other (farm cluster) should set up common ETP to avoid environmental pollutions. In areas where the water source is turbid with suspended particle, an intake reservoir for sedimentation is also very essential.

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BRACKISHWATER FINFISH CULTURE METHODS

G. Biswas

Historically, brackishawater fish culture appears to have developed in India, Indonesia, Philippines and some other countries of South and South-East Asia. In India, traditional brackishwater fish and shrimp farming known as 'Bhasabadha fisheries' in West Bengal and 'prawn filtration' in Kerala have been in vogue during last few centuries. These techniques were developed by skilled farmers of these regions for exploiting the naturally available fish and shrimp seeds. Recently, however, a system of fish and shrimp farming has been developed in specially designed brackishwater ponds. Today, brackishwater aquaculture also known as coastal aquaculture is mostly dependent on a single species, tiger shrimp, *Penaeus monodon*. Development of finfish culture in brackishwater has not been remarkable and it is sporadic. The present practices are based on the availability of natural fry and often several species are either purposely stocked or they gain entry with the tidal water, in spite of preventive measures. The most common species in brackishwater ponds are seabass, mullets, milkfish, pearlspot and catfish. Tilapia has also got entry to the coastal aquaculture. Here, the existing traditional and some improved methods of brackishwater fish culture are presented.

1. Traditional farming practices

Traditional method of aquaculture is practised in the extensive backwater systems of Kerala and Sunderbans mangrove swamps of West Bengal for a few centuries. Traditional fish/ shrimp farming is also done in a smaller scale in the coastal paddy fields in the states of Karnataka, Goa and Orissa.

The traditional prawn culture technique locally known as 'prawn filtration' is practised in low lying coastal paddy fields called *Pokkali* fields in Kerala. Both seasonal and perennial fields exist. Here, alongwith shrimp species some fishes are also encountered in these fields. Different fish species occurred are mullets, pearlspot, seabass, milkfish, threadfin bream, ten pounder, tilapia etc. Average yield from these fields is around 800-1000 kg/ha.

Brackishwater tidal wetlands, namely mudflats, swamps, marshes, paddy fields etc . situated 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 major sources of water supply for *bhery* fisheries are 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. 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 practiced 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 fishing 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 resenbergii*, *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. In this system production ranges from 500-1000 kg/ha/year. 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. Selective stocking is done in the *bheries* depending on the availability of natural seeds. Fish species cultured here are seabass, mullets and tilapia. Carps are also being used in low saline areas.

The traditional fish farming fields are known as *Khazan* lands in Goa. In *Khazan* fields fish/ shrimp farming is rotational with paddy culture. The average production from the traditional culture in Goa is about 350 kg/ha/year.

In Karnataka, paddy-cum-shrimp culture is practised in low lying coastal *Khar* lands. It is similar to prawn filtration practice of Kerala. Yield is about 400 kg/ha of which shrimps constitute 55% and the rest of the catch by fishes.

In Orissa, traditional practice of 'trapping and holding' locally known as *Ghery* has been in vogue for a long time in low lying areas and paddy fields. The average yield in the fields is 600 kg/ha/year.

2. Improved culture practices

The age old traditional methods of brackishwater fishes and shrimps still exist in several countries because of their simplicity and cheapness. These are being gradually replaced by modern improved culture practices.

2.1 Culture of Asian seabass, *Lates calcarifer*

As seed is regarded as the main critical input, so in the areas where wild seeds are available, the farmers go for improved farming system of seabass culture. Farmers generally follow polyculture or monoculture methods depending upon the availability of feeding materials.

2.1.1 Polyculture System

Polyculture 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 this improved polyculture method, the food required for seabass is produced in the pond itself and seeds are stocked thereafter. The pond for seabass polyculture is prepared first, following eradication of unwanted organisms and application of manures and fertilizers. Tilapia, *Oreochromis mossambicus* and *O. niloticus* which are omnivore and prolific breeders are the best suited candidates for polyculture with Bhetki as the primary crop. Tilapia fry as forage fishes are introduced first @15,000-20,000/ha, 1-2 months prior to seabass seed stocking. Tilapia are 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 3-5 g size are stocked @ 8000-10,000/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 upto 3 to 4 tons/ha is achieved.

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

2.1.2 Monoculture

Monoculture of *L. calcarifer* in ponds is a highly developed aquaculture industry in Taiwan. In India, it is practiced in some pockets where cheap trash fish as feed is available in

plenty. Seabass seeds are stocked @10,000-15,000/ha in well-prepared culture ponds. 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 them. 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 800 gm with a survival rate of about 60-70% and a production of 2.5 to 5 tons/ha is achieved. For the trash fish feeding, feed conversion ratio of 6-8 is obtained on wet weight basis.

2.2 Culture of other non-predatory fishes

It has been observed that monoculture of non-predatory brackishwater fishes is not as lucrative as mixed culture or polyculture systems. This is mainly due to low production in monoculture. Experimental monoculture of these fishes have been carried out since 1980s to till date to find out economically viable technology packages. However, farmers mainly practise the mixed or polyculture based on the natural seed availability. The combined culture of two or more species of fish and shrimp having compatible feeding habits is followed for increasing production from brackishwater farms. For polyculture in brackishwater, the selective fish and shrimp species should have the following characteristics:

- Hardiness and ability to tolerate considerable fluctuations in salinity and temperature
- Ability to grow fast in brackishwater ponds
- Ability to accept natural as well as supplementary feed
- High survival rate
- Good market demand

Based on the above criteria the following species of fish and shrimp are considered suitable for brackishwater fish culture:

Fish: Mullets- *Mugil cephalus* (Striped grey mullet)

Liza tade (Tade grey mullet)

L. parsia (Goldspot mullet)

Milkfish- *Chanos chanos*

Pearlspot- *Etroplus suratensis*

Shrimp: Tiger shrimp- *Penaeus monodon*

Before stocking of seeds, pond is prepared well following eradication of pests and predatory fishes, removal of bottom mud and liming, fertilization etc. The ready ponds are stocked

with seeds of fish species at 8000-15,000 nos./ha alongwith tiger shrimp seeds of 10,000-20,000 nos./ha. The stocking density varies with the quantum of seed availability. Natural pond productivity is maintained by fertilization. In addition, supplementary feed prepared from locally available ingredients can be used at 5-2% body weight. This kind of system can yield a total production of 1.0-1.5 ton/ha in 6-8 months.

Development of eco-friendly and cost-effective culture technologies of finfish targeting small-scale farmers is the need of the hour. Some steps towards brackishwater aquaculture development are extension of culture to inland saline areas, bringing more areas under culture, species diversification from existing shrimp to fishes etc. Availability of quality fish seeds will also help in expansion of culture.

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GROW-OUT SHRIMP FARMING AND MANAGEMENT MEASURES

P.S. Shyne Anand, A.Panigrahi and Sujeet Kumar

Introduction

Shrimp farming is one of the commercial activities in coastal areas of India. Presently, 1,57, 000 ha area is under farming with an average production of about 1.15 lakh metric tonnes of shrimp per year. The average productivity has been estimated at 700 kg per hectare per year. Grow out shrimp culture in India mainly ranges from traditional to improve traditional and extensive shrimp farming. Cultured shrimps contribute more than 70 per cent of the total shrimp exports from India. About 90 per cent of the shrimp farmers in the country have a holding of less than 2 ha, 6 per cent between 2 to 5 ha and the remaining 3 per cent have an area of 5 ha and above. In India modern shrimp farming started in mid 1980s. Shrimp culture resulted in the development of several ancillary activities such as seed and feed production, processing units etc. Together, these activities have generated employment, livelihood option and foreign revenue. The boom period of commercial-scale shrimp culture was in early 1990s and the bust came in 1995-96, with the outbreak of viral disease. The general ignorance of good farming practices has resulted in wide spread disease occurrence across the country. Now shrimp industry is reviving due to the adoption of good management practices and biosecurity measures. Major candidate species used for shrimp farming in India are *Penaeus monodon, P.merguiensis and P.indicus*. Shrimp farmers generally follow a one-phase production cycle in India, unlike two phase production cycle followed in some American and South East Asian countries. With the one phase cycle, the nursery ponds are eliminated, and the Post larvae are stocked directly into grow out ponds, after proper acclimation.

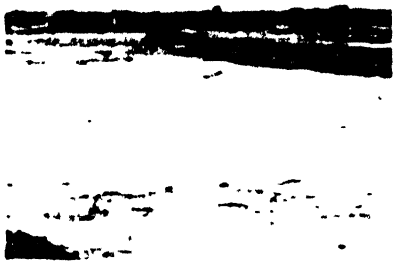
1. Pond preparation

1.1. Drying the pound bottom: Pond preparation is one of the most important pre stocking management measures essential for optimum growth of shrimp in grow out farming systems. It helps to reduce the risk of disease outbreaks and improve shrimp production.

1.1.1. Ploughing or raking: Ploughing or raking the pond bottom help to exposes the nutrient rich sub soil and fast mineralization and oxidation of the organic compounds and harmful gases. The pond bottom is allowed to dry for minimum one week till it gets cracks. Tiling and ploughing is not generally recommended in acidic soils as it increases the soil pH.

1.2 Top soil removal: The top black soil and bottom sludge to be removed to prevent development of anaerobic condition during culture period. The sludge must be disposed away from the pond site, so that it does not seep back into ponds. Grow out pond with high stocking density entire pond top soil is removed where as modified extensive ponds, areas of the pond where there is a high accumulation of organic matter from previous crops, such as feeding zone should be removed.

2.1. Liming: During pond preparation liming is applied to optimize pH and alkalinity conditions of soil and water. The type and amount of lime to be added depends mainly on the soil and water pH, which should be checked before lime application. The recommended levels of lime application during pond preparation are given in Table 1. The soil and water pH can be measured with a pH meter. Generally agricultural lime or dolomite can be applied if soils of pH >5and Quick lime or hydrated lime can be applied if soil pH below 5. Where disinfectants like bleach (calcium hypochlorite) is used then applies lime only 3-4 days after the application of disinfectant as lime reduce the effectiveness of the disinfectant.



Lime applied on pond bottom

Lime application in pond water

3. Water intake: Stringent measures to be followed to prevent entry and growth of any unwanted and pathogenic agents in culture ponds. It can be achieved via proper filtration of intake water using appropriate mesh screens, disinfection of intake water. Keeping a suitable reservoir also facilitate chemical treatment to reduce disease outbreak and to make water management more effective during production cycle.

4. Fertilization of pond water

4.1 Organic and inorganic fertilizers: The purpose of fertilization is to ensure the growth of primary producers in culture ponds. They initiate natural food web in the aquatic ecosystem and directly or indirectly contribute shrimp growth also. Moreover, it helps to maintain desirable level of transparency which prevents development of harmful benthic algae. Phytoplankton in culture ponds also help to improve the water quality parameters in grow out ponds. Organic fertilizer like dry cow dung at the rate of 500 – 2000 kg ha-1 and inorganic fertilizers like urea and superphosphate at 25 – 100 kg ha-1 can be applied depending on the

organic carbon content and available N (50-75 mg/100 g soil) and P(4-6 mg/100 g soil) content in the pond. Of the original dose, 10% can be applied fortnightly to maintain the desired level of algal bloom. The Secchi disc transparency should be in the range of 25-40 cm.

5. Stocking process

5.1 Seed selection: Healthy, pathogen free seed from registered hatcheries should be used for stocking. PL 15 or PL 20 are generally stocked in grow out shrimp ponds. The health status of the seed should be checked through standard procedures.

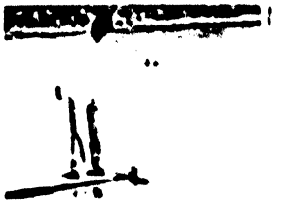
5.2 Acclimation: Careful prestocking measures to be followed as sudden fluctuation in water quality parameters induce stress in animal and it may result in their mortality PL can be acclimatized slowly to the pond salinity very slowly @ 3ppt/day. It is better to start the acclimatization at the hatchery itself. Acclimatization to temperature can be done by floating the seed bags for about 30 min in the pond before releasing larvae. Acclimatization to pH also to be done by mixing pond water with hatchery water in the seed bag

5.3 Stocking density: Stocking density generally depends on type of management followed. A maximum density of 6 nos/m^2 within the CRZ and a maximum of 10 nos/m^2 outside the CRZ are permitted by the Aquaculture Authority of India. Now days, high percentage farms are following low stocking densities in the country with high success rate.

6. Feed management: Proper feed management is crucial for profitability of shrimp farming as it forms 40-60 percent of the operational cost of the shrimp farming. The best shrimp feed will be a best expensive fertilizer if not managed properly. Feed management techniques are important in both improved traditional and extensive shrimp farming systems. Shrimp feed should contain 36-40% protein for good growth. Since shrimps are slow feeders, feed pellet should be stable in water minimum 2 hr without any disintegration. Recently, it has been reported that ideal shrimp feed should be consumed within one hour as delay in intake can nutrient leaching in aquatic system.

6.1 Feed size and feeding regime: Depending on the growth stage of the shrimp, size of the feed to be given varies. Their diameter varies between 1 mm to 2.5 mm and called as starter or granules (1-3 g shrimp size), Grower (3-15 g shrimp size) and finisher (15-35 gm). Feed can be given 2-10% of body weight of shrimp and % feeding rate decreases as shrimp size increases. Feeding frequency of shrimp is generally four times a day. As they are nocturnal in nature, maximum percentage of the total feed is given during evening or night hours.

6.2 Feed intake monitoring: Quantity of feed actually taken by the shrimp can daily noticed with the help of check trays. 4-6 number check trays can be used in one ha ponds. 1-2% of the total feed given can be kept in each check trays and can be measured after 1: 30 hr to 2 hr. Feed can be regulated proportionally by examining the amount of left over feed in the check trays. This helps to avoid under feeding and over feeding. Apart from feed monitoring and controlled feeding, it gives an invaluable source of data on what is going on in the pond helps in early detection of disease, and to maintain clean pond bottom and water free of deterioration.



7. Sampling schedule: weekly or fortnightly sampling is necessary to understand the growth and survival percentage of the cultured shrimp. Quantity of feed to be given is calculated based on average body weight and survival shrimp. Sampling can be done during early hours or evening with the help of cast nets.

8. Water quality management: Water quality management is vital aspects of shrimp grow out cycle. Water quality parameters must be regularly monitored to maintain parameters within optimum limits. Water quality parameters can be measured within the desirable limit via water exchange, aeration, periodical lime and fertilization application.

Optimum water quality parameters in culture ponds

Water quality parameters	Ideal range
Temperature(^{0}c)	28-33
Transparency(cm)	25-45
pH	7.5-8.5
Dissolved oxygen (ppm)	5-7
Salinity(ppm)	15-25
Ammonia -N	<0.01
Nitrate –N	<0.03

8.1 Water exchange: Water exchange is one of the easiest methods to ameliorate emergency situations arising in ponds such as algal blooms, high pH fluctuation above 0.5 in a day etc.

8.2 Aerators: Aeration is required to maintain optimum dissolved oxygen level in water i.e. above 5 ppm. Aerators introduce freshly oxygenated water, and are used at night and early in the morning when oxygen levels are at their lowest. Aeration is also provided during cloudy

days, rainy season algal bloom crash etc. Commonly used aerators in shrimp ponds are paddlewheel and aspirating aerators.

8.3 Liming and fertilization: Liming must be done when water pH drops below 7.5 and daily fluctuation is above 0.5 in a day. Maintenance dose 10% of original fertilizer to be added .Other chemicals like potassium permanganate, iodine compounds are also used in culture ponds.

9. Health management: Health status of the cultured shrimp should be monitored periodically and checked for their general health conditions, like body color, external/gill fouling, black gills, missing appendages, gut condition, and growth etc. Shrimp behavior and feeding trends should be monitored. Other protocols relating to pond treatment and cleanliness, and bio-security are additional developments which considerably reduce the chances of WSD spread. In case of any abnormality occur, remedial measures to be followed and an emergency harvest can be carried out. Before releasing the water to the drainage, treat the pond water with bleaching powder to avoid spread of disease into the neighboring farm.

9.1 Biosecurity measures: Migrating birds put droppings, dead or diseased animals and quickly consume shrimp in the ponds. Bird scares like noise cannon, plastic bags and glittering tapes can be used as safety measure. Pond fencing can also be done as a bioseurity measure to prevent intrusion of any animals in to farm.

10. Waste management: The discharge water from shrimp pond is rich in suspended matters (arises from uneaten feed, faecal matters, dead organisms) and dissolved nutrients (Nitrate, Phosphate). Discharge of this nutrient rich water in to adjacent lands and creek lead to sever negative implication on the environment. So, according to guide line issued by the Aquaculture Authority of India, Effluent Treatment Farm should be constructed if farm size is above 5 ha within CRZ and 10 ha above outside the CRZ.

11. Harvest and post harvest: Harvesting can be done by completely draining the pond either by gravity or through pumping. While harvesting, discharge water should be pumped in to settlement ponds before releasing in to open waters. Harvested shrimps must be immediately iced and transported in refrigerated to processing units without any delay in operation.

Suggested Readings:

Ravichandran,P and Pillai, S.M (2004). Hand book of shrimp seed production and farming,CIBA bulletin, No :16

Compendium of Acts, Rules, Guidelines and Notification, 2006. Coastal Aquaculture Authority.

SHRIMP POST-LARVAL QUALITY ASSESSMENT

R. Ananda Raja, Akshaya Panigrahi and Sujeet Kumar

Introduction:

Shrimp larval quality is considered as one of the key factors influencing the success of shrimp culture. Proper management in hatchery and nursery systems would ensure good survival and growth rate of shrimp post-larvae before stocking. In recent years, shrimp health management has become the main focus of improving production and minimizing infectious diseases in shrimp ponds. However, the ultimate goal of shrimp health management is to prevent the disease from occurring, reduce the incidence and severity of infectious diseases when they occur. To accomplish this goal, one should be concerned with the quality of post larval shrimp especially the selection of high-health one before stocking in the pond. Polymerase chain reaction exploits the capability of amplifying a small amount of genetic material from an organism, thus facilitates the detection of virus especially in carriers which normally contain a small amount of virus particles. Achievement of a good successful crop is not only dependent on the absence of shrimp pathogens but also relies on good physiological and morphological features. The PL with poor physiological and morphological features may show negativity to pathogens, but later may exhibit unexpected performance in terms of growth and survival resulting to poor production. So, this paper describes about the quality assessment of post larvae with respect to physiology and morphology.

I. Gross Examination:

It allows a preliminary assessment to be made on large number of post larvae (PL) simultaneously. An impression is gained on the over all activity and behavior of the PL. PL of the black tiger shrimp, *Penaeus monodon,* between the stages of PL 10 and PL 20 are selected and inspected before stocking. PL in the nursery tank should be inspected for size (too small, high variability in individual size/stage of development), number of rostral spines (PL 15-20 will have 4 to 6 spines), color (the presence of red or white pigment cells in the uropods gives the tail an open appearance, is a useful indication of the stage of development. If the uropods are not pigmented which may make the tail appear closed, then the PL are not sufficiently developed for stocking), uniformity, cleanliness (absence of fouling organisms), activity (active when fed, clinging to the sides of the tank), fullness of gut (long faecal strings can be seen projecting from the anus and loose in the water column), swimming behavior (consistently forward), number of PL and their distribution, etc. The advantage of this type of observation is that it is possible to observe a large number of PL in a short time. In most cases, this type of assessment is the only one carried out by the farmers. A beaker or bowl of

PL should also be sampled for closer inspection. With such small sample it is usually possible to observe the condition of the stomach to see if the shrimps are eating well, and to get a better idea of the state of cleanliness and any gross deformities present. Swirling the PL in the bowl to see if they are strong enough to maintain themselves and swim normally in a current helps to assess their strength. and tapping the side of the bowl to check their reactions (flicking response) are also useful in assessing state of health.

Stress Tests:

a) Formalin test:

Post larvae before stocking in grow-out pond are selected by exposing the animals to 150-200 ppm formalin for 30 minutes. After 30 min the PL that are still active or which move when prodded with a needle are counted and the result expressed as a percentage. Survival of more than 75 % is desirable. This is used to cull weak animals for WSSV and successful in reducing the number of infected PL stocked into ponds.

Survival percentage = (No. of active PL/Total no. of PL in beaker) X 100.

b) Salinity Shock:

This involves an exposure of the PL to 50% of the ambient salinity by taking a sample of water from the PL tank and diluting it 1:1 ratio with clean freshwater in one liter beaker. About 300 PL are taken from the tank and placed into the beaker, and after 3 hours, the PL that are still active or which move when prodded with a needle are counted and the result expressed in percentage. In this test, post larvae in good health have high survival (>75 %) rate.

c) Stress Hormone Test:

Some authors have tried using stress hormone such as corticosteroid to assess response of animal to induce stress using chemical. The test is experimental and would need further documentation to verify its usefulness.

If the inspector is satisfied with the condition of the PL following gross inspection, a sample of PL can be passed on to the lab for the microscopical examination. If the PL in the tank looks bad or unhealthy, then no microscopical analysis is needed to discard the whole population. The sample should be taken at random and contain at least 100 PL so that a further random sub-sampling can be done in the lab.

II. Microscopic Examination:

This helps to indicate quality related problems before they have progressed to a state sufficient to show up in the gross examination. For hatchery, this provides a useful early

warning so that corrective measures can be taken. For the farmer it brings an idea to accept or reject the PL although the PL appears good on gross inspection. If the small sub-sample examined contains unhealthy PL, it indicates that a large number of PL in the original population must be in deteriorating condition. On the other hand, if the entire PL in the sample passes, it does not necessarily mean that the whole population is in uniformly good condition. So, assessment method is one which is still to be refined.

The best time to take sample is mid-way between successive feeds so that the feeding state of larvae can be assessed. In the lab, the PL are placed in a beaker or basin and a sample of PL taken at random. Care must be taken to ensure that the sampling is not biased and especially to avoid selecting the PL which are easiest to catch. The PL are placed on a microscope slide for examination at 40x magnification. The number of PL placed on the slide depends on the size of the PL but is usually around five. At least 15 PL should be assessed per tank. Once the PL are on the slide, a quick inspection is made to check the chromatophore condition as this can change quickly while the PL are on the microscope due to stress (e.g. Evaporation increases the salinity of the water on the slide). The active PL will have distinct dark, red or blue spots in the tail. Following this, the other characters should be studied.

a) Back Muscle in 1-5 abdominal segments:

In healthy PL, It is clear with no striations or discoloration. Unhealthy PL shows discolored, striated, shrunken, cloudy and or grainy back muscle.

b) Tail Muscle in 6th abdominal segment:

A microscopic examination of the relative thickness of the ventral abdominal muscle and the gut in the 6th abdominal segment of the post larvae should be conducted to determine the muscle to gut ratio. This gives a useful indication of the nutritional status of the animal. High muscle to gut ratios (3:1) is preferable.

c) Hepatopancreas:

The hepatopancreas of the good grade PL will be full, dark, bubble-like or turbid appearance. Presence of the indigestible material or empty, shrunken and pale appearance of the hepatopancreas will indicate the poor status of the PL.

d) Deformities:

This can be again classified in to normal, slightly deformed and severely deformed. The PL should be carefully examined for the presence of appendages or body deformities, moulting problems and necrosis.

e) Fouling:

PL appears fuzzy due to fouling caused by some bacteria, fungi and protozoa. These will typically attach to the exoskeleton on the head and body and particularly around the head or legs or legs of the larvae.

f) Monodon Baculo Virus:

Squash mount of the hepatopancreas will show the occlusion bodies in the nucleus with malachite green stain.

g) *Vibrio* spp. Infection:

A presumptive diagnosis can be made based on the presence of bacterial plaques in oral region and appearance of "black balls" in the gut made up of poorly digested algae, melanized appendages tips or foci and motile rod-shaped bacteria in hemocoel.

e) Bolitas:

It is a syndrome involving the detachment of epithelial cells from the intestine and hepatopancreas, which appear as small spheres "white balls" within the gut. It is believed to be caused by bacteria and can be fatal.

Conclusion:

Along with the PCR and other molecular techniques for screening the diseases, this method of simple examination of PL would also help in optimizing the production.

Suggested Readings:

Better Management Practices (BMP) Manual for Black Tiger Shrimp (*Penaeus monodon*) (2005). Hatcheries in Viet Nam.

Manual on ASEAN good shrimp farm management practice. ASEAN Cooperation in Food, Agriculture and Forestry, Fisheries Publication Series No.1.

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FEED MANAGEMENT IN TIGER SHRIMP

Debasis De and T.K.Ghoshal

Shrimp farming has shown phenomenal growth in the last decade in India producing protein rich health food and earning valuable foreign exchange. Feed is a major input in shrimp farming. Preparation of nutritionally adequate feed for tiger shrimp (*Penaeus monodon*) involves understanding the dietary requirements of the species, proper selection of feed ingredients, formulation of feeds and appropriate processing technology for producing water stable pellet feeds. Depending upon the type of farming, a wide range of feedstuffs are used for feeding stocked shrimp. While no feed is used in traditional farming systems, supplementary and adequate feeds are used in improved extensive aquaculture.

The performance and success of a formulated diet for shrimp depends on many factors, the most important being

i) Feed formulation and nutrient content of feed ingredients

ii) Feed manufacturing process and physical characters of the feed

iii) Feed handling and storage

iv) On-farm feed management-feed application methods, feeding regime

v) Aquatic environment and natural food availability

To formulate a practical diet for tiger shrimp, first and foremost point to be considered is the nutrient requirement (Table-1) of the species. Shrimp diet should have adequate energy and protein to meet the requirement for maintenance and growth. In nature, shrimp can meet their requirement from a variety of feed available in the ecosystem. But when shrimps are cultured in confined systems, they should be provided with balanced diet as close to natural feed as possible.

Table 1. Requirement of major nutrients for tiger shrimp

Nutrient	Dietary requirement
Energy (Kcal/Kg)	2800-4300
Protein (%)	35-45
Lipid (%)	5-15
Carbohydrate (%)	20-25
Phospholipid (%)	0.1-2.0
Cholesterol (%)	0.5

Feed Management in culture pond

Feed management means control and use of feed for aquaculture operation in such a manner that utilization of feed is optimum with minimum wastage, negligible impact on environment, achieving best feed conversion ratio (FCR) and maximum growth of shrimp.

Proper feed management is essential for successful and profitable shrimp culture. As feed alone costs 50-55% of total culture expenditure, strict supervision on feeding of tiger shrimp is required. Following points should be strictly followed while feeding the shrimp for maintaining good pond hygiene and to reduce wastage of feed and to avoid accumulation in pond bottom.

1) Pond biomass should be assessed regularly and ration should be offered as per biomass of the pond.
2) Time and method of feeding should be proper. Daily ration should be divided and given 3 to 4 times a day (Table 4). The feeding activity and quantity of feed consumed may be checked by keeping feed in check trays (size: 80 cm x 80 cm)@ 4-6 nos./ha in different places in pond. After one month of stocking, consumption of feed should be checked by using check trays. 1% of feed ration is to be kept in check trays and observed after 2 hr. Depending on the quantity of feed consumed in the check tray, the next dose should be increased or decreased. Feed should be broadcasted evenly in a periphery of about six feet from dyke in all sides of the pond.

Table 2. Feeding Schedule for shrimp

Feed type	Shrimp weight (g)	Time of feeding				
		6.00 AM	11.00 AM	6.00 PM	10.00 PM	2.00AM
Starter	Up to 4.0	30 %		35%	35 %	-
Grower	4 – 15	25 %	15 %	30 %	30 %	-
Finisher	> 15	25 %	15 %	20 %	25%	15%

Table 3. Recommended shrimp pellet size

Feed type	Size of shrimp (g)	Pellet size
Starter	0-4.0	0.5-1.0 mm crumble
Grower	4.0-15.0	2 - 2.3 mm x 4 - 5 mm
Finisher	>15	2-2.5 mm x 6 – 8 mm

Shrimp appetite will vary due to the environmental conditions i.e., water quality, water temperature, sunny/overcast days and physiological conditions such as disease and moulting. Feed should never be given in excess as uneaten feed pollutes the water. As shrimps are the nocturnal feeder, larger doses may be offered in the evening and during night. Regular observations and experience helps in mastering the management of feeding in a culture farm. Generally during new moon and full moon moulting of shrimp takes place and they become sluggish and reduce the feed intake. So, quantity of feed offered should be reduced at the extent of 30-50 % during that period.

3) **Quantity of feed:** Generally the method of calculating the daily ration is based on the body weight of shrimp (Table 6).

Table 4. General guideline for calculating dose of feed

Days of culture	Expected survival (%)	Expected ABW (g)	% of ABW to be used as feed.	Feed Required (g) per 1000 post larvae/day	Total feed (g) required for the period
1-5	99	0.1	20.0	20	100
6-10	97	0.3	13.0	40	200
11-15	95	0.5	10.0	50	250
16-20	94	0.7	9.5	65	325
21-25	93	1.0	9.0	85	425
26-30	91	1.4	8.5	100	500
31-35	89	1.9	6.5	110	550
36-40	88	2.5	4.5	115	575
41-45	86	3.4	4.3	120	600
46-50	84	4.6	4.0	150	750
51-55	83	5.7	3.8	180	900
56-60	81	6.8	3.7	200	1000
61-65	79	7.9	3.6	225	1125
66-70	78	9.2	3.5	250	1250
71-75	76	10.5	3.4	280	1400
76-80	75	12.8	3.3	320	1600
81-85	73	14.9	3.2	350	1750
86-90	71	17.0	3.1	375	1875
91-95	69	19.2	3.0	425	2125
96-100	67	21.5	2.9	450	2250
101-110	65	24.6	2.8	425	4250
111-120	62	28.4	2.3	400	4000

Total feed required for initial stocking of 1000 pl. for culture of 120 days is 28 kg approximately. Success of feed management depends on the farmer's experience and observation on the feeding behaviour and feed intake of shrimp. Following a strict feed management, survivability up to 80 % and average weight of 30 g can be achieved in culture duration of 120 days. Progressive farmers may have small scale feed mill to prepare shrimp feed using locally available feed ingredients for tiger shrimp culture and may get a good economic return. Central Institute of Brackishwater Aquaculture (CIBA), Chennai and it's regional centre at Kakdwip extend technical guidance to set up feed mills in West Bengal and other parts of the country for preparation of shrimp feed using ingredients available in the country.

Suggested Reading:

Akiyama D.M and N.L.M. Chawng. (1995) Shrimp feed requirement and feed management. Aqua International, August- September, 1995. 14-27.

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HEALTH MANAGEMENT IN SHRIMP CULTURE

Sujeet Kumar, R. Ananda Raja and Akshaya Panigrahi

Introduction

Disease is a major constraint to shrimp aquaculture production worldwide. White spot disease, Loose shell syndrome, *Monodon baculovirus*, Vibriosis etc are major threats to shrimp industry. In a farm situation, a number of risk factors are responsible for outbreak of disease and its severity along with the primary cause i.e. pathogens. By adopting best managemental practices these risk factors can be minimized and outbreaks of disease can be prevented. The below mentioned measures can be adapted for avoiding the outbreaks and spreading of disease.

1) Seasonal factors and crop planning: Temperature is a crucial factor in disease outbreak, as its fluctuation stresses the animals. It was observed from field trial that farmers stocking earlier in the year, around February or March, had more chances of a successful harvest compared to stocking in May- June.

2) Pond preparation: Following measures should be adapted during pond preparation.

a. **Removal of bottom sludge**: This involves the removal of black soil layer which contain high organic content. It helps in maintenance of pH, and soil and water condition during culture period.

b. **Ploughing of soil**: It exposes the black soil layer to sunlight and atmospheric oxygen which oxidize the organic waste.

c. **Use of lime**: Liming during pond preparation optimizes pH and alkalinity conditions of soil and water. Quick lime or hydrated lime should be used if the soil pH is low *i.e.* pH <5 and shell lime or dolomite should be applied, If the soil pH is more than 5.

3) Pond filling and water preparation: Measures should be taken to remove the carrier host and pathogens.

a. **Water filtration**: This reduces the introduction of virus carriers such as crabs, wild shrimps and zooplankton and also avoids entry of fish or crustacean, which may be predator or competitor for shrimp.

b. **Use of reservoir**: For every two grow-out ponds one extra pond should be maintained as a water reservoir. Here, disinfection for removal of carrier and fertilization for growth of plankton can be ensured before final taking of water in grow out pond.

c. **Disinfection of pond water**: Using insecticides (should be selected which is not harmful to human and animal) and bleaching powder all life form in the reservoir should be destroyed. It reduces the risk of pathogens and carrier.

d. **Fertilization**: Use of organic fertilizer like dry cow dung reduces the risk of disease outbreak by reducing stress to post-larvae, and development of harmful benthic algae in the pond by preventing the sunlight touching pond bottom.

4) Seed Selection and Stocking: Large part of shrimp health depends upon the quality of postlarvae stocked in culture pond and its natural resistance to disease. Several managemental practices can be applied at this stage to reduce the risk of disease like:

a. **Rigid quarantine program**: Rigid quarantine program of 40 to 60 days should be scheduled for any exotic species introduced into the country for future culture.

b. **Use of specific pathogen free (SPF) and specific pathogen resistant (SPR) stocks**: SPF shrimp are of great value in disease free areas and SPR shrimp in disease endemic area. SPR shrimp is inappropriate for use in non endemic areas, as they may carry sub-clinical infections of the pathogen.

c. **Use of PCR tested broodstock and postlarvae**: White spot disease is tranismitted by contaminated water source as well as through infected broodstock. Therefore, only PCR tested WSSV negative broodstock should be used for spawning.

d. **Physical examination of post larvae in hatchery**: Before purchasing, shrimp post larvae should be checked for their general condition such as activity, color, size, *etc.* If there is any dead and abnormal colored PL in the tank, the entire batch should be rejected.

e. **Elimination of weak and dead PL**: Before stocking at the pond, PL should be treated with formalin at 100 ppm concentration for 30 minutes in well aerated tanks to remove weak PL.

f. **Stocking Densities**: Low stocking density reduces the disease outbreak.

5) Pond bottom and water quality management: Disease outbreaks in shrimp grow out culture is directly related to pond bottom and water quality. The following measures should be taken to maintain good quality soil and water in the grow out pond.

a **Water exchange**: Though water exchange at regular interval maintains the good quality pond water. But, after the outbreak of WSSV, zero water exchange (no water exchange) or minimal water exchange came into practice which reduced the incidence of disease outbreak by ensuring the biosecurity.

b. **Aeration** – Ponds using aeration tend to have higher shrimp production and low disease outbreak.

c. **Salinity and pH**: Salinity should be maintained below 15 and pH below 8.5. In high saline waters it is difficult to maintain water quality, especially a stable bloom which

make shrimp more susceptible to viral infection. In cases where pH exceeds 8.5, the toxicity of ammonia increases leading to higher stress conditions for shrimps.

d. **Use of chemicals like Lime, Zeolite, Benzal Konium Chloride (BKC) and Iodine**: Lime maintains soil pH while zeolite (a soil conditioner) helps in removal of toxic gases like ammonia. BKC is a strong disinfectant and kills bacteria.

e. **Bioremediation**: Bioremediation is a process of reducing hazardous wastes to environmentally safe levels through the use of microbes. Microbes such as *Bacillus subtilis and B. licheniformis* are used for removing bottom detritus. Nitrifying bacteria like *Nitrosomonas* and *Nitrobacter* are used on baggasse based substrate to reduce the NH_3 level. Anoxyphotobacteria are used for removal of H_2S from pond bottom.

6) Use of prophylactics and treatment in shrimp health management: Disinfectants, probiotics, immunostimulants, vaccine, drugs and antibiotics are employed as prophylactic and treatment measures in grow out culture and hatchery system.

a. **Probiotics**: Probiotics is a live microbial feed supplement which beneficially affects the shrimp by improving its intestinal microbial balance. Most commonly used probiotics in shrimp culture are Yeast, *Bacillus*, *Vibrio alginolyticus Lactobacillus acidophilus* etc.

b. **Immunostimulants**: Immunostimulant is a chemical or drug which enhances the non specific defense mechanism of the animals thereby impart better generalized protection. Many substances such as β-glucan, chitin, lipopolysacharide of gram negative bacteria, peptidoglycan of gram positive bacteria, lactoferrin, levamisole, and many nutritional factors and cytokine are used as immunostimulants.

c. **Vaccine**: Prophylactic vaccines may be used for controlling specific disease. Certain commercial vaccine against Vibriosis in shrimp is available. Vaccine development is underway against white spot syndrome virus using VP28 protein.

d. **Drugs and antibiotics**: The disease control measures using drugs would be useful only if they are applied during the early phase of the disease. Formalin, potassium permanganate, copper sulphate etc are used for ectoparasitic infection. Benzalconium chloride, iodine, oxytetracycline are used as bath treatment of bacterial disease and as disinfectant. There is a serious concern on the use of antibiotics, and their use in shrimp farming should be avoided.

7) Shrimp Health Monitoring: This include the regular sampling and check up of shrimp, soil and water quality, microbial status etc.

a. **Regular check up of shrimp**: Shrimps should be sampled once in a week by cast netting and should be checked for their general health conditions, like external appearance (body color, missing appendages, external/gill fouling, black gills or gill choking, *etc*), gut condition, and growth in terms of weight or length. Shrimp behaviour and feeding trends should also be monitored.

b. **Monitoring of soil and water quality parameter**: This includes monitoring of various physical parameter like temperature, salinity pH, total suspended solid etc. and chemical parameter such as ammonia, nitrate, nitrite, total organic carbon, dissolved oxygen, BOD, COD etc.

c. **Molecular tools in health monitoring**: Shrimps should be periodically examined for WSSV infection by PCR test. This will help in taking appropriate steps for disease control at very beginning.

8) Handling a Shrimp Disease Outbreak: Despite all the precautions, disease outbreak may occur. Prompt action is essential in such circumstances to rectify the problems, reduce the losses and minimise the impacts on neighboring farms. Under such circumstances, the following actions should be taken:

a. Check any abnormalities in water and soil condition and take immediate action to correct the problem.

b. Remove dead animals and bury them away from the ponds.

c. Emergency harvesting, if the mortality rate is increasing rapidly and shrimp are not feeding. It can be carried out preferably using cast netting to avoid discharge of infected water into the main water source.

d. Bleaching of pond water for 5 – 7 days before releasing into to the drainage.

e. Neighboring farmers should be kept well informed about shrimp disease problems, emergency harvesting and the time and date of water discharge.

f. The pond water should be treated in an effluent treatment system (ETS) before discharging to a common water source.

g. To avoid the cross contamination during periods of disease outbreak, surrounding farmers should try to avoid water exchange and should not use any equipment (nets, tanks, pumps, boat, *etc*) from affected farms.

h. To maintain water quality in the pond during such periods, feeding may be reduced. Liming should be done to maintain the pH above 7.5.

9) Farm Record Maintenance: It is a good practice to maintain all the records related to farm work. Records are necessary to identify problems in the pond environment and shrimp

health and to rectify these problems at the earliest during the production cycle. Record keeping also helps the farmer to learn from past mistakes, thus reducing risk and costs of production in subsequent crops.

Conclusion

Existing and emerging viral diseases is currently the single largest problem in shrimp culture. As of now vaccination or treatment is not available for control of such diseases. But, last ten years experience of shrimp disease management state that it can be controlled and its damaging effect can be minimized by adopting the best managemental practices. Special care should be taken during pond preparation, water intake and a good quality post larvae should be stocked. For maintaining good soil and water quality probiotics, bioremediation measures should be applied. Regular monitoring of shrimp health should be ensured and best measures should be taken in situation of disease outbreaks with the help of neighbouring farmer. Molecular techniques such as PCR can bring remarkable changes in disease control by earliest detection of disease.

Suggested Readings:

MPEDA/NACA. 2003. Shrimp Health Management Extension Manual. Prepared by the Network of Aquaculture Centres in Asia-Pacific (NACA) and Marine Products Export Development Authority (MPEDA), India, in cooperation with the Aquatic Animal Health Research Institute, Bangkok, Thailand; Siam Natural Resources Ltd., Bangkok, Thailand; and AusVet Animal Health Services, Australia. Published by the MPEDA, Cochin, India.

Subasinghel, R.P., D. Curry, S.E. McGladdery and D. Bartley. 2003. Recent Technological Innovations in Aquaculture. Review of the State of World Aquaculture, FAO Fisheries.

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NUTRIENT REQUIREMENT AND FEED FORMULATION FOR BRACKISHWATER FINFISHES AND SHRIMPS

T. K. Ghoshal and Debasis De

Protein and Amino Acid requirements

Like other animals fish and crustaceans require food to supply the energy that they need for movement and all the other activities that they engage in and the 'building blocks' for growth. However, they are 'cold-blooded' and as their body temperature is the same as the water they live in, they do not therefore have to consume energy to maintain a steady body temperature and they tend to be more efficient users of food than other farm animals. The food requirement of different species of finfish and shell fishes vary in quantity and quality according to the nature of the animal, its feeding habits, its size, its environment and reproductive state. Fish and crustaceans require food protein in the form of essential amino acids for maintenance of life, growth and reproduction and the requirement of protein depends on animal characteristics, i.e., species, physiological stage, size as well as dietary characteristics i.e. protein quality (digestibility and biological value), energy level etc. and also abiotic factors, i.e. temperature, salinity etc. The protein requirement of aquatic animals is higher than terrestrial animals which might be a consequence of the low energy requirements of ectothermic animals. Moreover the scarcity of carbohydrates and abundance of protein and lipids in the natural aquatic food web is also probably responsible for the common trend of aquatic organisms to use protein as an energy source. Protein is the most important and essential nutrient in the diet of shrimp and fish. The protein requirement in terms of dietary concentration (% of diet) is high. Protein is required in the diet to provide indispensable amino acids and nitrogen for synthesis of non-indispensable amino acids. A deficiency of indispensable amino acid creates poor utilization of dietary protein and hence growth retardation, poor live weight gain and feed efficiency. In severe cases, deficiency reduces the ability to resist diseases and lowers the effectiveness of the immune response mechanism. For example, experiments have shown that tryptophan deficient fish become scoliotic, showing curvature of the spine, and methionine deficiency produces lens cataracts.

Protein (amino acids) is used as a major energy source. Some economy can be made here if other dietary fuels are present in adequate amounts, e.g. increasing the lipid (fat) content of diet can help reduce dietary protein (amino acid) catabolism and requirement. This is referred to as protein-sparing effects of lipids. Protein requirement vary with the age of the fish and crustaceans. Younger animal generally require higher levels of protein (5-10% more protein) than older animals. Carnivores require high dietary protein (40-50%) than omnivores (25-35%). The protein requirement varies with size of shrimp and also with the source of

protein used in diet. The dietary requirement of protein for tiger shrimp *Penaeus monodon* ranges from 35 to 45% and for *Fenneropenaeus indicus* it ranges from 30-43%, which are the most important species for culture. It has been demonstrated that postlarvae and juveniles require higher protein in diet and the requirement decreases, as the shrimp grows larger in size. Among the brackishwater finfishes, requirement of protein for Asian seabass (Lates calcarifer), milkfish (Chanos chanos) and mullet (Mugil cephalus) is 40-45%, 40% and 35-40%, respectively.

Amino acids: The growth of fish and shrimp is directly related to the quality of protein in terms of amino acids. After digestion of protein, amino acids are metabolized at tissue level to form new proteins for growth, maintenance and energy. Protein in body tissues incorporates about 23 amino acids and among these, 10 amino acids must be supplied in the diet since fish and shrimps cannot synthesize them. These are termed as essential amino acids (EAA) and includes arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Amino acids are needed for maintenance, growth, reproduction and repletion of tissues. A large proportion of the amino acid consumed by a fish are catabolized for energy and fish are well adapted to using an excess energy in this way. It is found that if the amino acid composition of the protein in the feed matches with the amino acid composition of shrimp body tissue, such feed promotes good growth. Catabolism of protein leads to the release of ammonia.

Lipid requirement

Lipids (fats) encompass a large variety of compounds and a complex mixture of simple fat, phospholipids, steroids, fatty acids and other fat soluble substances such as pigments, vitamins A, D, E and K. Lipids have many roles: energy supply, structure, precursors to many reactive substances, etc. Phospholipids are responsible for the structure of cell membranes (lipid bi-layer). Fatty acids are the main active components of dietary lipids. Deficiency of essential fatty acid result in general in reduction of growth and a number of deficiency signs including depigmentation, fin erosion, cardiac myopathy, fatty infiltration of liver and 'shock syndrome' (loss of consciousness for a few seconds following an acute stress). The quantitative requirement of fat in the diet of shrimp is in the range of 5 to 10%. Fat levels of 6-8% are adequate in most of the fish diets. However, the quality of fat in terms of fatty acids is more important.

Fatty acids: Fish and shrimps are unable to synthesize fatty acids of the n-3 and n-6 series.and must be provided in their diets. Aquatic animals require higher n-3 fatty acids than terrestrial animals. Among aquatic animals, marine habitat require more HUFA than freshwater counterparts. Among the long chain fatty acids polyunsaturated fatty acids (PUFA) such as linoleic acid (18:2n6), linolenic acid (18:3n3), eicosapentaenoic acid

(20:5n3) (EPA) and docosahexaenoic acid (22:6n3) (DHA) are essential for growth, survival and good feed conversion ratio for *P.monodon* and other penaeid shrimps.The n3 fatty acids are more essential than the n6 acids. The fatty acids, EPA and DHA, which are known as highly unsaturated fatty acids (HUFA) of n3 series, are particularly important. Quantitatively EPA and DHA are needed at 0.5% and 1.0% in the diet of larvae and juvenile shrimp. Fresh water fish show requirement for n6 and n3 essential fatty acids (EFA), whereas marine fish show requirement of n3 and also HUFA. Studies in *Fenneropenaeus indicus* have shown that oils rich in PUFA such as fish (sardine) oil, squid oil and prawn head oil produce superior growth when incorporated in its diet. These oils are rich in HUFA. Marine fish oils are rich dietary source of n-3 series while plant oils are rich in n-6 fatty acids.

Phospholipids: Shrimp require phospholipids for growth, moulting, metamorphosis and maturation. Lipids of squid, clam, shrimp, fish and polychaetes are excellent natural source of phospholipids. The phospholipids, phosphatidylcholine (lecithin), is essentially required in the diet of shrimp for fast growth and good survival. Soya lecithin is a good source of phospholipid for shrimps. It is required at 2% level in the diet. The development and survival of larvae is significantly improved when the diet contains lecithin. Phospholipids are found to be involved in the transport of lipid, especially steroids in the haemolymph.

Steroids: Shrimps grow through the process called moulting and steroid hormones called, ecdysones, are responsible for moulting. To synthesize these hormones, the steroid cholesterol is required in the diet. Shrimps are not capable of synthesizing cholesterol in their body and hence must be supplied through diet. The requirement of cholesterol in shrimp diet was shown to vary from 0.5% to 1.0%. Cholesterol is not essential for finfishes. Many natural feed ingredients, such as prawn head waste and squid are good sources of cholesterol, which can be included in the feed formulations.

Carbohydrates requirement

The carbohydrate most commonly found in fish feed is starch, a polymer of glucose. Raw starch in grain and other plant products is generally poorly digested by fish. Cooking of the starch during pelleting or extrusion, however, greatly improves its digestibility for fish. However, even if the starch is digestible, fish only appear to be able to utilize a small amount effectively. Carbohydrates only represent a minor source of energy for fish. A certain amount of starch or other carbohydrates (e.g. lactose, hemicellulose) is, nevertheless, required to achieve proper physical characteristic of the feed. The nutritional value of carbohydrates varies among fish. Freshwater and warm water species are generally able to utilize higher levels of dietary carbohydrates than coldwater and marine species. Carnivorous fishes require less dietary carbohydrates level (<20%). Omnivorous and herbivorous fishes require high level of carbohydrates (40-45%). Carnivorous fish have poor ability to digest carbohydrates

due to low amount of amylase produced. The quantitative requirement of carbohydrate in the diet of shrimp is related to dietary protein and lipid levels. Depending upon the total energy content required in the diet, carbohydrate can be used from 10-40% level. Corn flour, wheat flour, tapioca flour and other grain flours are good sources of starch in shrimp feeds.

Vitamin and mineral requirement

Micro-nutrient such as vitamins and minerals significantly influence the growth and survival of fish and shrimp and these cannot be synthesized by these organisms. Even though, some vitamins such as niacin can be synthesized by number of animal's but are typically insufficient to meet physiological demand. Most of the vertebrates and some invertebrates are capable of synthesizing vitamin C (ascorbic acid) from glucose due to presence of enzyme gulonolactone oxidase whereas many finfishes and shellfishes cannot synthesize vitamin C due to absence of this enzyme. 'Black death' in shrimp is a classical symptom of vitamin C deficiency characterised by melanised haemocytic lesions distributed throughout the collagenous tissue. Hence, supplementation of vitamins and minerals become necessary for most aquatic organisms. The vitamin requirement depends on various factors such as size, age, growth rate, water temperature, composition of diets and environmental stress. Unlike higher animals, the recommended doses of vitamins for aquatic animals are higher, as many vitamins lost during the process of feed manufacture and also due to leaching. Vitamin deficiency symptoms in fish and shellfish are non-specific unlike in mammals. There are four fat soluble (A, D, E and K) and 11 water soluble (B and C) vitamins are required by various organisms. In crustaceans there is wide fluctuation in vitamin requirement studies and hence no standard vitamin premix has been evolved like in fishes and other vertebrates.

Table: Dietary vitamin requirements (mg per kg diet unless specified) for finfish and shellfish

Vitamin	Finfish	Shellfish
Vitamin A(IU)	2000-5000	30000-50000
Vitamin D (IU)	1000-2400	2000-2400
Vitamin E	30-100	30-40
Vitamin K	10-1000	4-6
Thiamine	1-15	15-30
Riboflavin	7-30	15-60
Pantothenic acid	25-50	60-120
Pyridoxine	3-20	60-120
Niacin	120-200	60-120
Folic acid	6-10	6-10
Cyanocobalamine	0.01-0.02	0.01-0.02
Choline	2500-3000	600-800
Ascorbic acid	50-70	8000-10000

Fish and shellfish can absorb minerals directly from aquatic environment through gills and body surfaces or by drinking. Hence, dietary requirement of minerals is largely

dependent on the mineral concentration of the aquatic environment. About 20 inorganic elements (macro and micro) are required to meet the metabolic and structural functions in the body of animals. The aquatic organisms regulate the mineral needs through dietary source and also through internal regulatory mechanisms in the kidneys and gills. In saline waters calcium (Ca) is abundant, which is absorbed by most aquatic animals. Since the availability of phosphorus (P) through water medium is poor, P should be made available through diet. Usually the preferred Ca:P ratio is 1:1 in feeds of aquatic species. Mono and dicalcium phosphate contain more available P than tricalcium phosphate. Incorporation of P should be very discrete in fish and shellfish feeds, as most of it gets excreted leading to eutrophication. The dietary requirement of P ranges from 0.5-0.9% in fishes and 1-2% in shellfishes. The requirement of magnesium (Mg) in shrimp and fish ranges between 0.04-0.3%.The requirement of zinc (Zn) ranges from 15-30 mg/kg diet for fishes and 80-120 mg/kg diet for shellfishes. The requirement of iron (Fe) ranges from 150-200 mg/kg diet for fishes and 60-100 mg/kg diet for shrimps. Major deficiency symptoms of manganese (Mn) in fishes are cataracts and abnormal curvature of the backbone and malformation of tail. A dietary supplementation of 11-13 mg/kg restores normal growth in fishes. In shrimps, the requirement goes up to 40-60 mg/kg which may be due to periodic ecdysis.

Trace minerals like copper (Cu), cobalt (Co), selenium (Se), iodine (I) and chromium (Cr) have some role in general upkeep of the organism. Their dietary incorporation enhances growth and survival. Copper is needed by crustaceans because of hemocyanin. Optimum dietary level of Cu ranges from 40-60 ppm and it was also observed that omission of Cu from the diet was not detrimental as, crustaceans are able to meet their demands from seawater.

Feed Formulation

Before proceeding with formulating a feed, the ingredients are to be selected from available sources. No single ingredient can be expected to provide all the nutrient requirement. Each ingredient in the diet should be included for a specific reason i.e., either to supply a specific nutrient or physical property to the diet. Formulation of a feed by the nutritionist is only the beginning of a process that ends when the feed is finally consumed. Feed formulation is essentially a recipe making process keeping in mind the nutritional requirement of particular species, palatability and growth promoting ability of that feed. These objectives can be achieved by judicious selection of feed ingredients, mixing them in proper proportion and presenting them in a most acceptable form.

The basic technique used in ingredient selection is through "Least cost" or "Best buy" calculations

Least-cost or Best-buy technique

The price of the feedstuffs used in diet formulations must be considered to formulate a cost-efficient diet. Feedstuffs can be compared with one another on the basis of cost per unit of protein, energy, or amino acid. The cost of protein is often the greatest part of the cost of a fish diet. Therefore, substantial savings can be made by using best-buy techniques to determinate least expensive protein supplement.

When several feedstuffs are available to supply a particular nutrient then it is useful to calculate the cost per unit of nutrient from each of the ingredients and compare.

Example: If soybean cake costs Rs.16/kg and contains 45 % protein-

Cost/ kg protein = 16/0.45 = Rs.35.56

Ground nut cake costs Rs. 13/kg and contains 40 % protein

Cost/kg protein = 13/0.40 = Rs.32.50

Thus, although soybean cake contains higher level of protein, the cost per kg protein from ground nut cake is less. Therefore ground nut cake is a better buy.

To compare feedstuffs on the basis of cost per unit of an amino acid, one can calculate the best buy in the same way as before.

For example, sesame oil cake which has twice as much methionine content as does groundnut cake on a per unit protein basis would be a more attractive buy at comparable prices.

These kinds of comparisons are only valid if the nutrient in one feedstuff is as valuable or available to the animals as the same nutrient in another feed. Such comparisons should be made whenever prices charge.

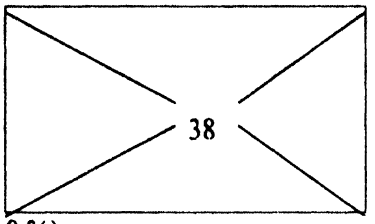
Balancing nutrient levels

In most animal diets, protein is the most expensive portion and is usually the first nutrient that is computed in diet formulation. The energy level of the diet is then adjusted to the desired level by addition of high energy supplements which are less expensive than protein supplements. The square method is an easy way to determine the proper dietary proportions of high and low protein feedstuffs to add to a feed to meet the dietary requirement of the animal to be fed. The protein in the diet can be adjusted by following Pearson's square method. For example to prepare a diet with 38% protein using soybean meal (CP – 45 %), fish meal (CP-55%) and wheat flour (CP-10 %), ingredients are to be divided into two groups- GroupA- protein rich ingredients (soybean meal and fish meal) and group B- energy rich ingredient (wheat flour). Mean protein percent has to be calculated from both the groups. A square is constructed first and the names of the feed groups are written on the two

left corners along with the mean protein content of each group assuming that under each group ingredients are mixed in equal proportion. The required protein level of feed is written in the middle of the square. Next, the protein level of the feed is subtracted from that of the ingredients and answer is placed ignoring the positive or negative sign.

Group A (CP-50 %) Group A 28

Soybean meal : Fish meal (1:1),
Mean protein=(45+55)/2=50 %



Group B (Wheat flour-CP-10 %) Group B 12

40

Add the figures on the right hand side of the square, i.e., 28+ 12 = 40

Now to make the feed with 38 % protein we should mix

Group A ingredients- 28/40 x 100 = 70 %

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

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

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

The square method is helpful to novice feed formulators because it can get them started in diet formulation without the need to resort to trial and error. The square method can also be used to calculate the proportion of feed stuffs to mix together to achieve a desired dietary energy level as well as a crude protein level. The square method cannot be used to simultaneously solve for both crude protein level and ME level.

Linear Programming

The mathematical technique available to nutritionists for selecting the best combination of feed ingredients to formulate diets at the least possible cost is linear programming. The informations necessary for feed formulation using linear programming are

1. Nutrient content and DE or ME of ingredients;
2. Unit price of feedstuffs including vitamin and mineral mixtures;
3. Any other additives to be used in the feed; and
4. Minimum and Maximum restriction on the amounts of each ingredient in the feed

Least-cost linear programming software for diet formulation is readily available, the price varying with the sophistication required. A commonly used spreadsheet a such as Lotus 1-2-3 can also be utilized for formulating feeds, incorporating a smaller number of variables.

It should be noted that least-cost feed formulation is not always practical for small scale aquaculturists using on-farm feed manufacture facilities where are the choice of ingredients available is limited.

Quadratic programming formulation

Nutrient requirements used in linear programming feed formulation are fixed usually for maximum rate of growth. This may not be the best decision from economic point of view. Nutrient constraints may be relaxed to bring down feed cost while still achieving acceptable lower growth. Quadratic programming takes into account the growth response within a range of nutrient constraint. Therefore, good understandings of biological response functions from actual feeding trials are essential in the use of quadratic programming. For example, it was reported that inclusion level of arginine could be reduced by 20% with only a 5% likely reduction of growth of Nile tilapia.

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FEEDS AND FEEDING MANAGEMANT IN BRACKISHWATER FISH CULTURE SYSTEM

Debasis De and T.K.Ghoshal

Production of aquaculture is, in fact of the fastest expanding agricultural industries in the world, with annual growth rates in excess of 30 percent per year. Output increased from a level of 2.9 to 4.6 million tones between 1990 and 2000. Production of 3 million tones of farmed marine / diadromous finish/shellfish species (wet basis) in 1995 would have required over 1.5 million tones of fish meal and fish oil (dry basis) or the equivalent of over 5 million tones of pelagic fish (wet basis; assumes a pelagic to fish meal conversion factor of 5:1). This is not surprising as fish-meal and fish oil usually constitute 50-75 percent by weight of compound aqua feeds for most commercially farmed carnivorous finish species and 25-50 percent by weight (together with shrimp meals and squid meal) of compound aquafeeds for marine shrimp. Hence, many feed ingredient alternatives to fishmeal at varying levels are being sought in order to attain sustainable aquaculture in the current millennium. Preparation of nutritionally adequate feed for fish and shrimp involves understanding the dietary requirements of the species, proper selection of feed ingredients, formulation of feeds and appropriate processing technology for producing water stable pellet feeds.

The performance and success of a formulated aquafeed depends on many factors, the most important being

- Feed formulation and nutrient content of feed ingredients
- Feed manufacturing process and physical characters of the feed
- Feed handling and storage
- On-farm feed management-feed application methods, feeding regime
- Aquatic environment and natural food availability

Feed Management in fish culture systems

Feed management means use of feed in such a way that utilization of feed is optimum; wastage is minimum thereby negligible impact on environment, achieving best feed conversion ratio and maximum growth and production of fish and shrimp. A very good quality feed can produce poor result if the feed management is poor, whereas, a moderate feed can produce very good results under good feed management.

The foremost critical factor is selection of appropriate feeds and planning of optimal feeding regimens. Suitable feed should fulfill the nutritional requirements of species under culture. Proteins, lipids, carbohydrates, vitamins, minerals and water are the six major classes of

nutrients, which are used for building, maintenance of tissues and supply of energy. The requirement for these nutrients varies depending on the species according to their feeding habit, habitat in which they live in and the stage in their life cycle. Our aim should therefore be to produce nutritionally balanced feed with optimum protein energy ratio. It should also ensure that nutrients are not lost in water during the feeding process. Therefore, aquaculture feeds of different formulations are processed using the special technologies to ensure the diet remains intact in water before ingestion. and that should nutrients are prevented from dissolving. These general categories of feeds used in aquaculture are wet feeds with moisture contents of 50-70 percent. semi moist formulated feed with moisture contents of 20-40 percent and dry pelleted feeds with moisture contents of less than 10 percents. Since problems are associated with the distribution, handling, utilization, storage and quality of wet feeds and moist feeds, more and more dry feeds are manufactured either by steam pelleting or by extrusion pelleting. Advances in fish feeds and nutritional studies mean that many commercial feeds satisfying a wide range of options are now available.

Following points should be strictly followed while feeding the fish for maintaining good pond hygiene and to reduce wastage of feed and to avoid accumulation in pond bottom.

1. Pond biomass should be assessed regularly and ration should be offered as per biomass of the pond.
2. Time and method of feeding should be proper.

Ration size

The size of daily food ration, the frequency and timing of meals are the key factors influencing the growth and feed conversion. Hence, the optimal feeding regimens must be determined as per the feeding behaviour, appetite and functioning of the digestive systems and the various specific chemical substances, which act as feeding stimulants for fishes. Fish lose weight when their food intake falls below that required for maintenance. When ration size increases, the growth rate increases. Generally the method of calculating the daily ration is based on the body weight of fish. The quantity of ration varies from 100% of body weight for larvae and fry and gradually reduced to 50 %, 20%, 10%, 5% and 2-3% as the fish/shrimp grow marketable size. Ration size is also estimated by various methods using the feeding charts, feed equations, growth prediction and check try etc. Besides the food ration size, the optimal food particle size also affects the growth and feed conversion efficiency. Large fish can ingest small particles, but it requires more energy to capture the required equivalent weight or smaller food particles. This results in measurable reduction in food conversion

efficiency. Attention should also be given to the influences of feed shapes, colors and textures of pellets on ingestion rates.

Feeding methods

Production of high quality fish at least-cost depends on an effective feeding method. Various techniques exist, from hand feeding to mechanized feeding. They depend on diverse range of factors such as labour costs, scale of farming, species under farming, the type of holding system and hatchery or grow out systems. Often farmers use a combination feeding methods such as hand feeding to mechanized feeding. Feed bag suspended at different places in ponds is most common method of feeding to the fish. In mechanical feeding system, demand feeder is used in which fish approaches to the feeder for its feed requirements when they feel hungry. It was observed that fish quickly learn how to obtain feed. The growth of fish is good with best FCR and minimum wastage of feed in self-demand feeding system. This method works best with finfish farming. A reliable and least- cost feeding system should ensure the effective distribution and spread of adequate feeds in aquaculture ponds.

Schedule and frequency of feeding

The total feed required in a day should not be fed at a time. Scheduling and frequency of feeding greatly help in successful feed management. Time schedule for feeding the fish may be fixed in such a way that larger ration may be given when the fish is expected to be most hungry. If night feeding is limited the morning feeding should have larger ration. There should be a minimum of three time schedules of feeding in a day- morning, noon and evening. Species which are having nocturnal feeding habit should get comparatively larger portion of the ration in the evening/night. Frequent feeding of small portion of ration help in better utilization of the feed and thereby lead to efficient FCR. There must also be a mechanism in each case to monitor the feed consumption and offering of next dose of feed should be regulated on basis of consumption from the previous feed offered.

Handling and storage of feeds

Optimizing handling and storage procedures on farms is an essential component of good management practice. High quality feed can readily spoil and denature if stored under inadequate conditions or for too long a period. In correctly stored feeds may not only be unappetizing to fish or lacking in essential nutrients but also may contain toxic and antinutritional factors. This can lead to abnormal behaviour, poor feeding response and growth. Hence different feed types such as wet feeds, moist feeds and dry feeds must be handled and stored under appropriate conditions.

Water quality

The interrelationships between feeding and water quality in aquaculture is complex. By providing optimal species-specific requirements such as temperature, dissolved oxygen, pH and salinity, adequate feeding to satiation, improved growth and survival can be ensured. When the water quality parameters fall below optimal levels, feeding and growth will be impaired and the species under culture will be stressed accumulation of left over feed together with excretory products is associated with high BOD, NH_3, H_2S, CH_4 and harmful effects of eutrophication. This is a critical issue in management since effluent quality can be linked directly to feeds and feeding practices and is regulated under water pollution control laws in many countries. Thus, feeding regimes should be designed to minimize the nutrient loss and faecal output and to maximize the nutrient retention and health status of the cultured fishes. Judicious feed management is an important factor in achieving good feed efficiency and reducing wastage. Selecting feeds, which are freshly prepared, quality assured and proven with best potential FCR, could reduce waste production. Poor quality and water stable feeds, which have lost their nutritional potency and are poorly accepted by the fish, should be rejected. Appropriate particle size of the feed should be designed for a particular stage. The ration size and feeding schedules should be regulated with reference to feeding guides, response of fish and environmental conditions.

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ROLE OF SOIL AND WATER PARAMETERS IN BRACKISHWATER CULTURE PONDS

G. Biswas and P. S. Shyne Anand

Suitable bottom soil condition and high quality water are essential ingredients for successful pond aquaculture. Water quality management has been considered as one of the most important aspects of pond aquaculture for many years, but less attention has been given to the management of pond bottom soil quality. It is the fact that the condition of pond bottoms and the exchange of substances between soil and water strongly influence water quality. So, at present the practical aquaculturists put more emphasis on management of pond soil rather than water quality. As healthy pond ecology provides suitable environment for the cultured animal to grow properly, maintenance of optimum culture environment through soil and water quality management limits the risk of stress and diseases of species under culture.

1. Soil parameters

The role of bottom soil in determining productivity of a pond is well understood. The production of various primary food organisms depends largely on the availability of different nutrients. Dynamics of availability of most of these nutrients, in turn, is determined by the condition prevailing in the bottom soil. Considering this significance, bottom soil is designated as the chemical laboratory of the pond. However, suitable soil quality problems are common in aquaculture ponds, and therefore, many methods are used for the purpose of improving pond soils.

1.1 Soil texture

Soil texture indicates the relative proportion of soil particles, viz. sand, silt and clay. Many important physico-chemical properties influencing the fertility of fishponds are affected to a great extent by soil texture. An ideal pond soil should not be too sandy to allow water seepage or too clayey to keep all the nutrients adsorbed on to it. Therefore, brackishwater soils with moderately heavy texture such as sandy clay, sandy clay loam, clay loam, silty loam are found to be favourable for brackishwater aquaculture.

1.2 Soil reaction (pH)

Soil may be acidic, alkaline or neutral. Soil pH is one of the important factors for pond productivity point of view, as it controls most of the chemical reactions in the pond environment. Near neutral to slightly alkaline soil pH (7 and little above) is considered to be ideal for fish production. Too low soil pH can reduce the availability of key nutrients in the water and lower pond productivity.

1.3 Organic carbon content

Organic carbon acts as a source of energy for bacteria and other microbes that release nutrients through various biochemical processes. Pond soils with less than 0.5% organic carbon is considered unproductive while those in the range of 0.5-1.5% and 1.5-2.5% to have medium and high productivity, respectively. Organic carbon content of more than 2.5% may not be suitable for fish production, since it may lead to excessive bloom of microbes and oxygen depletion in water.

1.4 Carbon to nitrogen ratio (C:N)

C:N ratio of soil influences mineralization process by microbes. Mineralization is very fast, moderately fast and slow at C:N ratios in the range of less than 10, 10-20 and more than 20, respectively. In general, soil C:N ratios between 10 and 15 are considered favourable for aquaculture and a ratio of 20:1 or narrower gives good results.

1.5 General nutrient status

Nitrogen, phosphorus and potassium are the major nutrients present in soil for phytoplankton production. Generally, small amount of potassium is needed in fish ponds. Single most critical nutrient for pond productivity is phosphorus content of soil and water. Pond soils with 30, 30-60, 60-120 ppm and more than 120 ppm available phosphate (P_2O_5) are considered to have poor, average, good and high productivity, respectively. Ponds with less than 250 ppm available soil nitrogen are regarded as low productive, while concentrations in the range 250 to 500 ppm and above 500 ppm are considered to be medium and highly productive, respectively.

2. Water quality parameters

Water quality parameters influence the pond environment. The pond environment should be optimum for the species to be cultured. Therefore, it is important to know about water quality parameters and their management, which affect the growth and survival of aquatic organisms.

2.1 Temperature

Temperature affects the fish metabolism by molecular dynamics and biochemical reaction rates. Warm water species in brackishwater system grow best at temperatures between 25^0C and 32^0C. The chemical and biological processes in a pond ecosystem are doubled for every 10^0C increase in temperature. This means that aquatic organisms will use twice as much dissolved oxygen at 30^0C as at 20^0C and biochemical reactions will progress twice as fast at 30^0C as at 20^0C.

2.2 Dissolved oxygen (DO)

Though DO is a critical parameter in fish culture, generally this variable has less influence in brackishwater farms, but farms with high density culture need artificial aeration using paddle wheel aerators. The optimum DO content of pond water is in the range of 5 ppm to saturation level for good growth of fish.

2.3 Salinity

Salinity is the total amount of solid material in gm contained in one kg of seawater, when all the carbonate has been converted to oxide, the bromine and iodine replaced by chlorine and all organic matter completely oxidised. Salinity of brackishwater ranges between 0.5 to 30 ppt. Salinity influences the solubility of different dissolved gases. Fishes and shrimps are highly sensitive to sudden changes in salinity. Cultured species living at a particular salinity should not be placed in water with lower or higher salinity without proper acclimatization.

2.4 pH

pH is a measure of hydrogen ion concentration in water and indicates how much the water is acidic or basic. Water pH affects metabolism and physiological processes of fish. pH also exerts considerable influence on toxicity of ammonia and hydrogen sulphide as well as solubility of nutrients and thereby water fertility. Optimum pH range for culture environment should be 7.5-8.5. Liming and applying gypsum ($CaSO_4$) are done to increase and reduce the pH, respectively.

2.5 Turbidity

It is caused in ponds by several factors including suspended soil particles, plankton population and humic substances produced through decomposition of organic matter. In culture systems, turbidity caused by planktonic organisms is a desirable trait, whereas that caused by suspended clay particle is undesirable. It is measured by Secchi disc transparency. Optimum range of transparency in a culture pond is 25-45 cm. If it is less than 25 cm, water treatment is required and if it is more than 45 cm, fertilization is done to increase plankton production.

2.6 Total alkalinity

Alkalinity of water is determined by all the carbonates and bicarbonates of alkaline and alkaline earth metals present in solution. The most common basic ions which are indispensable for fish culture are calcium and magnesium. Water containing alkalinity more than 40 mg/l as $CaCO_3$ is found to be productive.

2.7 Carbon dioxide

High concentration of carbon dioxide can be tolerated by fishes and shellfishes and most species will survive in waters containing upto 60 mg/l. Its concentration in water increases during night hours and eutrophic condition due to phytoplankton die-off. Application of CaO and $Ca(OH)_2$ can remove excess carbon dioxide.

2.8 Ammonia

Ammonia is a product of fish metabolism and microbial decomposition of organic matter. Fish are very sensitive to unionized ammonia (NH_3) and the optimum range is 0.02-0.05 ppm in pond water. Normally in the case of high DO and high carbon dioxide concentrations, the toxicity of ammonia to fish is reduced.

2.9 Nitrite

Under normal conditions, the nitrite concentration of fish ponds is negligible, as the ponds are kept well oxygenated. It is an intermediate product in the bacterial nitrification of ammonia to nitrate. Nitrite is highly toxic to fish as it oxidises haemoglobin to methemoglobin, which is incapable of transportation of oxygen. Optimum level of nitrate is less than 0.2 ppm.

2.10 Hydrogen sulphide

Under anaerobic condition hydrogen sulphide is produced in pond bottom soils and it is highly toxic to fish. At concentration of 0.01 ppm of hydrogen sulphide fish lose their equilibrium and subjected to sub-lethal stress. Frequent exchange of water can prevent building up of hydrogen sulphide. Further, increasing water pH through liming can also reduce the hydrogen sulphide toxicity.

Proper pond management is the key to sustainability in aquaculture, and enhancing sustainability of pond aquaculture can improve soil and water quality and reduce the volume and pollution potential of pond used water. Proper procedures for pond management will improve environmental conditions, sustainability and profit.

Suggested Readings:

Water Quality Management for Pond Fish Culture by C. E. Boyd. Elsevier Scientific Publishing Co.

Soil and water quality management in brackishwater aquaculture. CIBA Special Publication No.13, 2001.

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ROLE OF MICROALGAE IN AQUACULTURE

P.S. Shyne Anand, Sujeet Kumar and A. Panigrahi

Introduction

Aquaculture all over the world depends on the production and use of microalgae as live food for commercially important fin fish and shell fishes. Depending on the life stages, the micro algae are consumed either directly (mainly herbivorous fishes) or indirectly via phytoplankton – zooplankton food web. They are the primary producers of the aquatic ecosystem and food resources primary consumers. Apart from their role in aquatic food web, many microalgae are commercially important and possess vast potential in the manufacture of pharmaceuticals, health foods, dyes, fertilizers and biofuels. Commonly used microalgae and their various roles in aquaculture system are described below.

Commonly used microalgal groups in aquaculture systems

- Chlorophyceae : *Choleralla* sp , *Dunaliella* sp, *Haematococcus pluvialis*
- Bacillariophyceae: *Chaetocerus* sp, *Skeletonema* sp ,*Thalassiosira* sp *Phaeodactylum* sp , *Nitzschia* sp ,*Navicula* sp , *Cyclotella* sp
- Cyanophyceae : *Spirulina, Anabaena* sp ,*oscilatoria* sp ,*Lyngbia* sp
- Prymnesiophyceae : *Isochrysis* sp ,*Pavlova* sp
- Prasinophyceae : *Tetaselmis sp.*
- Eustigmatophyceae*: Nanochloropsis sp.*

1. Water quality improvement: The phytoplankton communities improve the physicochemical parameters in a culture pond by utilizing as the dissolved nutrients and carbon dioxide for their growth and photosynthesis. They can be used as biological indicators of water quality associated with culture systems as they are sensitive to changes in water quality. A good picture of the current conditions in the ponds can be derived by looking at plankton indicators such as their biomass, abundance and species diversity. Phytoplankton provides desirable level of transparency which protects the cultured organism from temperature fluctuations, and prevents benthic algal growth.

2. Natural food source in aquatic ecosystems: Phytoplankton communities are primary producers of aquatic ecosystem and form the basis of food web. They are the direct food source for primary consumers like zooplankton and herbivorous fishes. They are indirectly involved in the growth of secondary consumers also. Predation on zooplankton by fishes or shrimps transfers a significant proportion of the nutrients from natural biota to their tissue.

3. Live food organism in hatcheries: Massive culture of selected strains of live microalgae is used in fin fish and shell fish hatcheries for larval feeding. Most of the microalgae the

richest source of essential nutrients and poly unsaturated fatty acids. In shrimp hatcheries, algae are added during the non-feeding nauplius stage so that algae are available immediately upon molting into the protozoea stage. Algal species most often used in shrimp hatcheries are *Chaetoceros calcitranus, Skeletonema costatum* and *Tetraselmis* sp etc. Intensive rearing of bivalve larvae is solely depends on the continuous supply of many micro algae like *Isochrysis* sp., *Tetraselmis, Pavlova* sp. In hatcheries, microalgae are used either in live or concentrated freeze dried form. Moreover, the polysaccharides present in the algal cell walls stimulate the non-specific immune system in the larvae. In fin fish hatcheries, apart from their role in nutritional enrichment of live prey organisms such as *Artemia* and rotifers, algae are often used directly in the larval rearing tanks to generate green water systems. Major algae used for this purpose are *Nanochlopsis* sp, *Chlorella* sp., *Tetraselmis* sp., *Isochrysis* sp etc. The effects of the presence of micro-algae in the larval rearing tank helps to stabilize the water quality in static rearing systems (remove metabolic by-products, produce oxygen).

4. Nutritional benefits of microalgae: Commercial utilization of micro-algae is economically viable, and there is a worldwide market for algal derivatives. They have recently received a lot of attention from aquaculture industries as sources of proteins, lipids, minerals, poly unsaturated fatty acids, polysaccharides, carbohydrates, vitamins and antioxidants. For instance, many eukaryotic microalgae are well known protein and β-carotene sources which have gained commercial success. For instance, astaxanthin and carotenoids can be naturally produced from *Haematococcus pluvialis* and *Dunaliella salina* respectively. Micro algal derivatives are also used in human or animal food industry as feed supplements, and are commonly called as single cell proteins. *Spirulina*, a cyanobacterium is widely used as protein (above 70%) and vitamin B12 supplements. Microalgae like *Nannochloropsis* sp, *Phaeodactylum tricornutum and Nitzschia laevishas* proposed as an alternative source of essential polyunsaturated fatty acid mainly EPA, DHA. Screenings of microalgae have revealed that several species produce alpha-tocopherol in concentrations higher than conventional foods traditionally considered as rich sources of this vitamin. A good example is *Euglena gracilis, Dunaliella tertiolecta* and *Tetraselmis suecica*

5. Role in waste water management: Now a days micro algae are widely used in waste water treatment due to their ability to utalize various dissolved nutrients in highly eutrophied pond system. For instance, In shrimp farm discharges, where N:P ratio is 1.1 to 6.7 , micro algae are found to be excellent in removing disolved Nitrogen and Phosphorous from water. The use of algae for sewage oxidation is also found to be increasing as energy prices increase and more knowledge about the available waste water treatment technologies. Studies show

that microalgae like *Scenedesmus* sp, *Ankistrodesmus* sp and *Chlorella* sp are found to be efficient waste water treatments due to their fast growing nature. Oxygen production by microalgae for waste oxidation is generally recognized. There are also promising results demonstrating algae contribution in enhancing sedimentation, removal of organic contaminants, heavy metals and organic toxins. Studies show that they can biodegrade many hazardous organic pollutants. Microalgae also act as an interesting raw material for the production of biogas and biofertilizers.

Mass scale Production of algae: Large scale commercial production of microalgae is possible with the help of industrial process like photobioreacters. Mass scale production of algae for rearing of fin fish and shell fish larvae can be achieved in indoor and outdoor units by inoculating the pure culture of micro algae in to the system. In hatcheries and research laboratories, number of conventional media such as, F/2 media, Walne's–Conway, Scheiber's, Miquel media etc are used for culture of algae. In aquaculture ponds natural phytoplankton can be generated by adding inorganic or organic fertilizers which provide required Nitrogen and phosphorous for its growth.

Challenges: Even though large-scale cultivation of algae is possible, a great deal of research needs to be done to increase our understanding of the species, optimal growing conditions, and characteristics of the products that can be derived, and to lower costs of production. Lack of control over the growth and composition of algal population often result in high fluctuation and the blooming of unwanted and toxic species of blue green algae and dinoflagellates in aquaculture ponds. This results in mortality of cultured fishes, oxygen depletion, eutrophication problems etc.

Suggested Readings:

Burford, M (1997). Phytoplankton dynamics in shrimp ponds. *Aquaculture Research* 28:351–360

Munoz, R and Guieysse, B (2006). Algal–bacterial processes for the treatment of hazardous contaminants: A review *water research* 40: 2799 – 2815

Olaizola, M (2003).Commercial development of microalgal biotechnology: from the test tube to the marketplace. *Biomolecular Engineering* 20:459- 466

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HATCHERY TECHNOLOGY FOR ASIAN SEABASS SEED PRODUCTION

G. Biswas

Asian seabass, *Lates calcarifer* known as Bhetki in northern and eastern India is one of the important brackishwater cultivable species. This fish shows high growth rate and is cultured commercially in Australia and south-east Asian countries. It fetches high domestic market price (Rs.120-200/kg) and has considerable export destinations. In India, mainly traditional culture by entering tidal water carrying prey fish and shrimps into ponds is followed. Non-availability of adequate numbers of seeds is hindering expansion of seabass monoculture in the country. CIBA, Chennai has developed and standardised the technology package for year-round seed production of seabass which could be popularized to meet up the seed demand.

1. Biology of seabass

Seabass is predatory carnivore, feeding mainly on small crustaceans and fishes in nature. In absence of food, they show cannibalistic behaviour. It grows fast in fresh and brackishwater attaining 1-2 kg within a year. Adult fishes migrate towards sea for breeding. Sexes are separate, but difficult to differentiate. Seabass is protandrous hermaphrodite fish. Majority of individuals from early age groups (2.0-3.5 kg) are males, but when they attain 4 kg and above (4 years old), the majority of them become females. Seabass spawns during April to November in Indian waters. Spawning takes place in the sea in shallow areas of 5-10 metre water depth. After hatching, larvae are drifted with tides to estuarine areas and juveniles spend their growing stage in brackishwater zones.

2. Seed production

For establishment of a complete seabass hatchery following infrastructures are necessary: a) Broodstock holding tanks, b) Maturation tanks, c) Spawning tanks, d) Hatching tanks, e) Larval rearing tank, f) *Artemia* hatching tanks, g) Live feed culture tanks, h) Algae culture unit, i) Nursery tanks. Saline water is collected either from tidal affected areas or from sea using bore well. Water is stored in reservoir and filtered through biological filter, rapid sand filter and sometimes U-V ray is also used for purification

2.1 Broodstock development and management

The adult and sub-adult fishes can be procured from wild catch or farm and cage reared stock. Mature fish weighing 2-10 kg are selected as brooders, among which smaller fish are males and bigger ones are females and these fishes are made fully mature for

breeding within 6-months. Fishes from distant places have to be transported using vehicles with water holding facility having inlet and outlet provisions and an inner lining of materials such as foam, so that the fish will not get injured during transportation. Before transferring these brooders to broodstock holding tanks/ maturation tanks, they are acclimatized and observed closely for 3-5 days.

2.1.1 Stocking in maturation tanks

Fishes are stocked at 1 kg/ m^3 in 100 ton capacity concrete tanks. One tank (100 ton) can hold 10 females of 6 kg body weight and 16 males of 2.5 kg body weigh.

2.1.2 Water quality management

Broodstock fishes maintained in captive condition should be provided with required environmental conditions for maturation and spawning. The desirable water quality parameters for broodstock development are:

Temperature	28-32^{0}C
Salinity	28-33 ppt
pH	7.0-8.2
Dissolved oxygen	> 5 ppm
Ammonia	< 0.1 ppm
Nitrite-N	< 0.01 ppm
Phosphate	< 10-20 ppm
Suspended solids	< 2-5 ppm

Water should be clear and it is filtered through biological filter or pressure filters to get desirable quality. The tank bottom and sides are cleaned regularly and 70-80% water exchange is done daily.

2.1.3 Feed management

Fresh low cost trash fishes like tilapia, sardines, horseshoe mackerel etc., can be procured, cleaned and packed in polythene bags of 2-4 kg and stored in deep freezer at -20^{0}C. At the time of feeding, stored fishes can be taken out, thawed, washed, chopped and fed to the fishes at 5% of body weight. Under captive condition, fishes are weaned slowly to inert diet before starting of this kind of feeding. Excessive feed should be used to avoid deterioration of water quality and unused feed should be removed immediately.

2.1.4 Health care

Regular health checking and proper prophylactic treatment are done. To avoid any infections broodstock tanks are disinfected once in 3 months. Healthy gravid fishes can be obtained in 6-8 months from well maintained broodstock fishes.

2.2 Selection of spawners

The size of mature females will be 4-7 kg and males 2-3 kg. The males will ooze milt if the abdomen is gently pressed. The females should have eggs with diameter more than 0.45 mm. The gonadal condition is assessed by ovarian biopsy using a polythene cannula of 1.2 mm diameter. Brood fishes selected for induction of spawning should be active, free from disease, wounds or injuries.

2.3 Induced spawning

Since spawning in seabass is influenced by lunar periodicity, the days of new moon or full moon or one or two days prior or after these days are preferred for induction of spawning. For induced breeding of seabass Luteinizing Hormone Releasing Hormone analogue (LHRH-a) marketed by SIGMA Chemicals. USA is used. The dose of hormone injected has been standardized as a single dose of LHRH-a at 60-70 µg/kg body weight for females and 30-35 µg/kg body weight for males. To ensure proper fertilization normally two males are introduced for one female in the spawning tank. After injection, the fishes are released in 10-15 ton capacity spawning tanks. Fishes injected with LHRH-a hormone respond for spawning after 30-36 hours of injection. Prior to spawning gradual swelling of the abdomen will be seen indicating the ovulation process. At the time of spawning the fishes will move faster male and female together and milky white substance is seen at the water surface. There will be a fishy odour which can be felt from few metres away. Seabass is a protracted intermittent spawner (releasing eggs batch by batch). It has high fecundity and in one spawning fish may release 1.0-3.0 million eggs. The process of spawning will continue during subsequent day also. The externally fertilized eggs are transparent and are of 0.75-0.80 mm size floating on water surface. The unfertilized eggs are opaque and slowly sink to bottom. In natural spawning of seabass in good maturity condition, fertilization rate will be 70-90%.

2.4 Incubation and hatching

Fertilized floating eggs from spawning tank are collected following one of three methods, viz. overflow method, scooping/ seine net collection method and siphoning method. For overflow and scooping methods bolting net clothe of 150-200 µ mesh size is used. The collected eggs are washed first to remove debris and transferred to hatching incubation tanks of 200-250 litre capacity with cylindro-conical shape. Eggs are kept at 100-200 nos./litre density facilitated with continuous aeration. Water temperature of 27-28°C is desirable. The eggs hatch out in 17-18 hours after fertilization. The freshly hatched out larvae are of 1.4-1.6 mm size and are transferred to larval rearing tanks.

2.5 Larval rearing

Freshly hatched healthy larvae from the incubation tanks are transferred carefully to the larval rearing tanks (LRTs). LRT can be circular or rectangular FRP or concrete tank of 4-5 ton capacity and these tanks are stocked with larvae at 40-50 nos./litre. Depending on the age and size, the larval density is reduced to 20-25 nos./litre on 10th day and after 15 days, the density is maintained around 10-15 nos. litre. After yolk absorption, rotifer (*Brachionus plicatilis*) is given as larval feed from 3rd day post hatching. Initially rotifer is maintained at 20 nos./ml in the larval rearing tanks. From 4th to 15th day the rotifer concentration is increased to 30-40 nos./ml gradually. Green algae (*Chlorella, Tetraselmis, Isochrysis*) as food for rotifer is maintained at 20,000 cells/ml in the LRTs. *Artemia* nauplii are given as feed along with rotifers and green water from 10th day at 2000 nos./litre. From 16th onwards the larvae are exclusively fed with *Artemia* nauplii at concentration of 4000-6000 nos./litre. From 25th day the larvae can be fed with *Artemia* sub-adult (biomass) along with cooked minced fish/shrimp meat. Then the fry can also be weaned slowly to artificial feed. To maintain water quality in the LRTs, 30-40% water change is done daily. Differential growth is found in seabass growing stage and the larger ones known as 'shooters' may prey on smaller individuals resulting in low survival. To avoid this grading should be done once in three days from 15th day or whenever differential growth is seen in LRTs.

2.6 Nursery rearing

Seabass fry of 25-30 days old of size 1.0-1.5 cm are reared further in tank or net cage known as *hapa*. Circular or rectangular RCC or FRP nursery tanks of 5-10 ton capacity are stocked with fry at 500-1000 nos./m^3. The fry are fed 3-4 times with cooked minced fish/ shrimp meat with 1.5-2.5 mm particle size at 100-20% body weight daily. At present, formulated larval feed is also being used. Grading is necessary to carry out once in three days. Water exchange at 70% is followed on daily basis. *Hapas* of 2x1x1 m size made up of nylon webbing are fixed in ponds and used for nursery rearing. Fry are released at 200-500 nos./m^3 in *hapas*. After 30-35 days of rearing in tanks or *hapas* seeds attain average body weight of 1.5-2.0 g with 60-80% survival rate.

Suggested Readings:

Improved hatchery technology for Asian seabass *Lates calcarifer* (Bloch). CIBA Special Publication No. 34, 2008.

Report on training course on Seabass Spawning and Larval Rearing, 1-20 June 1982, Thailand. FAO Corporate Document Repository.

POND BASED NURSERY REARING OF SEABASS

G. Biswas

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.

Suggested Readings:

Improved hatchery technology for Asian seabass *Lates calcarifer* (Bloch). CIBA Special Publication No. 34, 2008.

Seabas Hatchery, Technology Series published by SEAFDEC.

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LARVAL NUTRITION OF SHRIMP AND FINFISHES

T. K. Ghoshal and Debasis De

Introduction

Larval nutrition in finfish and shellfishes had been the most studied because of its commercial importance as against other species. The requirement of nutrients varies throughout the life cycle of an individual. Start feeding or larval nutrition is critical whenever candidate species is evaluated. At early stages the requirements of nutrients is comparatively high which declines with age which is related to the basal metabolic rate. Also the requirements depend upon the feeding habits that change accordingly to the morphology of digestive organs and processes. Despite a simple gut the larvae need sufficient food to grow several folds during this period. Nutritionally adequate feed hold clue to successful larviculture. Efforts are directed to solve several issues in larviculture in aquaculturally advanced nations. Larviculture nutrition, particularly first feeding by early larval stages, appears to be the major bottleneck for industrial upscaling of aquaculture of fish and shellfish. Generally, finfishes and shell fishes larval nutrition mainly depends on two processes: (1) Morphological processes and (2) Digestive processes

Morphological processes

Studies have shown that there are diversified morphological differences in larval and adult stages. The first and foremost differences are the mouth size changes which affect the capacity of the fish/shellfish to ingest food. Success of survival ability of the organism depends on the selection of particle size in accordance with the mouth size. Certain species of fish and shellfishes are unable to even feed on live *Artemia* nauplii or even nauplii of rotifer which are smaller (95-350μm). Apart from the size of the mouth in larval satges, the digestive tract morphology has significant role in determining the specific feed requirement. The digestive tract is simple, relatively short gut and epithelial gut lining shows absorptive enterocytes with many microvilli on their luminal surface with few secretory cells. With the onset of exogenous feeding, marked morphological changes take place in the larvae. There is gradual initiation of mucosal fold and development of regional differentiation in the intestinal region. Micromolecules (protein/lipid) of nutrients are absorbed by pinocytosis and nanomolecules are intercellular broken down and absorbed. Majority of the candidates used in mariculture except salmon have very limited yolk reserves at hatching, mostly lasting for not more than one or two days. At first feeding they still have small mouths, often with an opening of less than 0.1 mm. In shrimp larvae feed size is not the only problem; but larvae

pass through different larval stages changing from a herbivorous filter feeder to a carnivore. In shellfishes, the larvae subsist on embryonic food initially. Subsequent larval stages depend on the filtering mechanism mostly phytoplankton-marine algae (*Chaetoceros sp.*, *Skeletonema sp.*, etc.) depending upon their preferential feeding status. Later larval stages start feeding on live organisms (Rotifer or *Artemia sp.*) by holding like the adult forms.

Digestive process

It is well known that in early phase of larval development, the secretion of digestive enzyme is limited because most of the epithelial cells are absorptive. Once the exogenous feeding starts the digestive tract morphologically develops and enzyme secretion initiates. In case of most fishes, the enzyme activities depend on the development of gastrointestinal tract. Certain enzymes like trypsin level increases upto day 12 and decreases to day 16. Thereafter it increases to day 25. Similarly, pepsin which is active at low pH increases from day 16 onwards. However, enzymes like chymotrypsin and amylase activity largely remains unchanged in fishes throughout the larval development. Thus, the relative gut length, gut passage rate, tryptic activity and the ability to reabsorb digestive enzymes in the hind gut-all increased with age from larvae to adults stage. It has been established that proteolytic activity increased when exogenous proteases from live prey contribute in the stomach of larvae. Studies on seabass (*Lates calcarifer*) larvae shows that the exogenous proteolytic enzymes and endogenous trypsin secretion induced by the ingested live food is sufficient to cause rapid breakdown of rotifers. Considering all these drawback in digestion mechanism in larval cycle, development of larval diets which are species specific with better efficacy in terms of maximum nutrient availability at minimum loss is one of the challenges faced by the nutritionists World wide.

Nutrient requirements

Larval nutrient requirements are not yet defined completely. However, it is expected to match the composition of yolk and a pre-feeding fish has a broader set of nutritional requirements which is indicated by the proximate composition of eggs of the fishes. Protein is the most abundant component, which resides mainly in the yolk. Lipid is the major constituent which varies widely. Lipids, mainly form the structural components of cell membranes and is also used as energy. Triglycerides and wax esters also meet energy requirements. It is also reported that carbohydrates are utilized to the maximum extent between fertilization and hatching. Current knowledge centres around yolk utilization, survival and growth based on certain essential nutrients like essential fatty acids (EFA) mainly the highly unsaturated fatty acid (HUFA) of the omega 3 and omega 6 series, which

are critical for first feeding fish larvae. Moreover free amino acid depletion in yolk indicates its use in energy metabolism apart from being employed at different rates in protein synthesis.

Lipids: are indispensable in early stages of fish life. Lipids are the main source of energy from gastrula stage in marine fish embryos. Omega 3 HUFA's are required for the normal growth and survival of larval fish. Mortality and deficiency signs such as underdeveloped swim bladder are reported in larval fish grown on low levels of omega 3 HUFA's. Eicosapentaenoic acid (EPA) 20:5 n-3 is the most essential, because it is a constituent of cellular membranes and several developing tissues. It was also found that the larval fish requires relatively large amounts of exogenous EPA. Further, inclusion of phospholipids, mainly phosphatidyl choline (lecithin) and phosphtidylethnolamine (cephalin) in larval feeds improved their growth and survival. Though a precise role is not known, it is supposed to be involved in formation of new cell components and it is also known that the rate of biosynthesis of these compounds fails to meet the developmental requirements. Live foods like rotifers and artemia are used to study this aspect by manipulating the level of these components in them through bioencapsulation / nutrient enrichment / nutritional boosting/ metabolisation. With these conclusions, it was stressed that novel feed technologies should aim to achieve the aformentioned levels of essential fatty acids, inositol and choline in the larval diets. In spite, of the inherent limitation *n*-3 HUFA's in artemia, enrichment of artemia nauplii with commercial fish oils should he discouraged and speciality oils such as tuna-orbital oil and highly purified preparations should be encouraged. Single cell speciality triacylglycerol oils, new marine fish phospholipid resources from fishery by-products and mainstream fisheries have to be further explored for effective larval nutrition.

Proteins and amino acids: Freely soluble amino acids occur in high amounts in the egg yolk, it is reported that free amino acids represented 50% of the total osmolality in the newly spawned egg. Predominant amino acids detected in fish eggs were leucine, valine, alanine, lysine, isoleucine and serine. The common yolk protein is known as phosvitin. A decline in the free amino acid concentration occurs as a result of metabolic turnover within the embryo, particularly for protein synthesis. However, taurine and phosphoserine did not decline. Constant amounts were present throughout the embryonic development without a clear role. Studies with

microparticulate diets indicate that the essential amino acid composition of the fish body closely matches its dietary requirements.

Diets for larval fishes

Until larvae start feeding on organisms, the cellular growth and energy needs of developing embryo are met by nutrients within the egg. The natural habitat of larval growth is a supermarket of microscopic life forms. Larval shrimps or finfish in their native habitats cannot afford poor nutrition. Aquaculturists take great pains in hatchery feeding protocols to generate live feeds. Current technology allows for the "in house" culture of several live feeds including artemia nauplii, rotifers and the monoculture of live micro algae (eg. *Skeletonema, isochrysis, chaetoceros, chlorella* etc.). Other live feeds have included diverse species such as ciliate protozoa, copepod nauplii, planktonic invertebrate larvae, trocophores and veligers. Given the constraints of economy and time, hatchery culturists have attempted to replicate the natural environment for larval rearing. The majority of hatcheries rely upon live culture of algae, rotifers and other microorganisms along with feeding *of artemia* nauplii. Such live feeds provide either precursors or molecules in finished form, which cannot be synthesised within the culture species. Scarcity of planktonic prey organisms and nutrients in turn, when the larva commences exogenous nutrition will result in body tissue autolysis and eventual death. The key question is whether the mix of *artemia* nauplii and cultured microalgae/microorganisms is adequate to simulate the wild larval nutrition and overcome the constraints of intensive larval rearing? Is there a sufficient supply of building block amino acids derived from the protein inputs, developmental biochemicals and immuno-chemicals to provide the basis for survial? What is the role of synthetic or conserved diets? The aforementioned queries invoke the ultimate question of live versus prepared diet? The answer lies in the compromise of nature and technology. This includes specially formulated synthetic and conserved natural diets. New technologies including lyophilisation of wild organisms and spray drying of microalgae and particulates, provide non-invasive preservation technology.

Criteria for selection of live food organisms

Physical qualities looked into are purity and availability and acceptability of the food organism selected. The nutritional indicators are digestibility of the organism in question and the bioavailability of nutrients/energy. Other considerations include the ease in procurement, reproducibility of the organism and the economy of the whole

culture cycle using the chosen organism. Micro algae from the primary link in the food chain and 20 different species of diatoms and flagellates in the size range of 2-20 μ is indicative of the physical size of the food organism. Rotifers fall within the size range of 50-200 μ and artemia within 200 – 500 μ. The magnitude of the size and the numbers for example is that 3000 live prey per fish is required to complete a 30 day larval phase and the food consumption in terms of body weight can be as high as 74.3% as daily ration. Unlike the larger size groups, larval nutrient requirements had been more elusive for most culturists. Therefore, most larval feeds available for various species are not complete replacement for live feed but effectively are used as supplement. The natural live feed has an array of nutrients available which enables the cultured organisms to obtain its entire nutrient requirement without supplementation.

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BRACKISHWATER FINFISH AND CRUSTACEAN DISEASES AND THEIR CONTROL

Sujeet Kumar and R. Ananda Raja

Introduction

Aquaculture represents the fastest growing food sector industry in the world. Diseases are among the greatest deterrents in this momentum. Fish and shellfish encounter diseases from all bio-aggressors such as viruses, bacteria, parasites and fungi. Out of this viral disease are the biggest threat to aquaculture industry, and livelihood and food security of common populace. In this chapter, some of the important diseases concerned in shrimp and fish culture are discussed.

Diseases of Brackishwater Finfish

The major disease problems which affect brackishwater finfish culture are Viral nervous necrosis, Iridovirus, Epizootic ulcerative syndrome and Vibriosis.

Viral disease in fish

Viral Nervous Necrosis: Viral nervous necrosis or Viral encephalopathy and retinopathy is a devastating disease of many species of marine fish cultured worldwide.

- Causative agents: Single stranded RNA virus of the genus beta-nodavirus.
- Transmission: Direct transmission by influent water, utensils, vehicles etc., vertical transmission from infected spawners to fry, and horizontal transmission between sick and healthy fish occur.
- Clinical signs: Spiral or looping swim pattern, swim bladder hyperinflation, wasting.
- Lesion: Vacuolation in the grey matter of brain and retina of eye, intracytoplasmic inclusion in nervous cells, nervous necrosis of the spinal cord, brain and retina.
- Diagnosis: Based upon sign and lesion, virus isolation in SSN-1 cell culture, indirect florescent antibody test (IFAT), PCR and ELISA.
- Prevention and Control: Measures like identification and culling of carrier broodfish, stress reduction at spawning time, chemical disinfectant of eggs, influent water and larval tank between batches should be ensured as no vaccine or treatment method is available.

Iridovirus infection: Iridovirus is the causative agents of serious systemic diseases in many brackishwater and marine fishes. The disease is caused by double stranded DNA virus of genera Lymphocystisvirus and Ranavirus. *Ranavirus* causes systemic disease in infected fish and are associated with high morbidity and mortality. Histological lesions in affected fish

include enlarged basophilic cells in the gill, kidney, heart, liver, and spleen. Recently real time PCR has been developed which shown improved rapidity, sensitivity, reproducibility, and the reduced risk of carry over contamination over normal PCR. An antibody based enzyme-linked immunosorbent assay (ELISA) has also been developed to detect iridovirus infection.

Bacterial disease in fish

Bacterial diseases occur in nursery, rearing and grow out ponds causing severe economic losses to fish farmers. Vibriosis is a major disease occurring in marine and brackish water fish and characterized by hemorrhagic septicemia. The causative agent of Vibriosis is the *Vibrio anguillarum*, and other Vibrio sps. Some other brackishwater bacterial pathogens are *Nocardia spp, Streptococcus agalactiae, Streptococcus iniae*, etc. Several methods are being used for the control of bacterial disease. Many antibiotics, sulphonamides, chemicals, disinfectant, herbal preparations etc. are available for preventive as well as curative measures. Many killed vaccine with long duration of immunity are also available for preventive the disease occurrence.

Fungal Disease in fish

Epizootic Ulcerative Syndrome: The disease is reported in freshwater as well as brackishwater fishes throughout India.

- Causative agents: The disease is caused by non septate fungus *Aphanomyces invadans*. Disease is invariably associated with secondary infection by gram negative bacteria and rhabdoviruses.
- Transmission: Zoospore is the infective agents which are transmitted through water, direct contact between fishes, and transport of infected fishes into new area.
- Lesions: Hemorrhagic ulcers on head which often extend to skull leading to exposure of brain.
- Clinical signs: Sudden high mortality in wild and farmed fish with different types of fish gets affected and showing abnormal behavior. Large ulcers and deep erosion of tissue may be seen in more chronic form.
- Laboratory diagnosis: Detection of non septate, branching fungal hyphae in the periphery of the lesion, presence of extensive severe mycotic granuloma and distinctive flocculant necrotic muscle fibres. Invasive fungus extend through muscle tissue to spinal cord, kidney, and peritoneum.

- Prevention and control: Destruction of infected fish, disinfection of contaminated equipment, drying and liming of pond before restocking, reduced feeding during outbreak are the preventive measures. Lime should be added to the pond at 400-600kg/ha.

Protozoan parasites in fish

They are either external or internal parasites. Dinoflagellates, *(Amylodinium)*, Ciliates (*Cryptocaryon, Trichodina*), Myxosporeans, *Microsporidium* are major parasitic disease in fishes. *Amylodinium* and *Cryptocaryon* is an external parasites which is usually attached to the gill filaments or body surface of the affected fish. Microsporidian and Myxosporean are internal parasites and infect various internal organs. The affected fishes are treated with short bath of 200 ppm formalin for 1 hour or copper sulphate bath (0.5 ppm) for 3-5 days with aeration and daily water exchange.

Shrimp Diseases

With the rapid increase of production, the sector experienced parallel increase in quantum of diseases. In last two decades many disease and its causative agents have been identified which lead to search for innovative means of control.

Viral disease of Shrimp

Viruses are ultramicroscopic, infective agents capable of multiplying in the host living cells causing improper cell function or cell destruction leading to the death of the host. Viral diseases constitute the most serious threats to shrimp industry due to its high infectivity, pathogenicity and total lack of curative measures. Following are the important viral diseases affecting shrimp industry:

White Spot Disease: White spot disease is the most lethal disease to the penaeid shrimp industry worldwide.

- Causative agent: Double stranded DNA virus called white spot syndrome virus of genus Whispovirus and family Nimaviridae.
- Transmission: Water and by cannibalism of weak or moribund shrimp, vectors, transovarian infection by affected broodstock.
- Signs and symptoms: White spot over cuticle, decreased feed consumption or cessation of feeding, lethargic or moribund shrimp accumulate at pond surface, erratic swimming behavior, and overall body colour often reddish.
- Diagnosis: It can be identified presumptively by white spot over body. Tissue impression smears of cuticular epidermis, gills, lymphoid organ and gut wall show

extensive damage and viral inclusions. It can be rapidly identified by polymerase chain reaction (PCR), western blot, and in situ hybridization. Bioassay of filtered tissue homogenates could also be used to confirm the viral etiology.

- Prevention and control: So far, no treatment is available. Only integrated management involving PCR screening and elimination of infected broodstock from the hatchery, stocking of ponds with post larvae negative for WSSV by PCR, elimination of potential carriers, continuous removal and destruction of moribund and dead shrimp could be used as preventive measure. Till now no vaccine is available.

Yellowhead Disease:

- Causative agent: Single stranded RNA virus of genus Okavirus, family Roniviridae of the order Nidovirales.
- Transmission: Contaminated water, cannibalism of weak or moribund shrimp, animate vector, net and other equipment. Vertical transmission has not been found.
- Signs and symptoms: Yellow coloration over dorsal cephalothorax, initial period of high feed consumption followed by cessation of feeding, moribund shrimp accumulate at the pond surface and edges with slow and erratic swimming behavior, overall body colour abnormally light or bleached appearance.
- Diagnosis: Histologically, infected shrimp show massive systemic necrosis and basophilic cytoplasmic inclusions in tissues of ectodermal and mesodermal origin. It can also be detected by reverse transcriptase polymerase chain reaction (RT-PCR), western blot assay, and In situ DNA hybridization.
- Prevention and Control: Proper cleaning and disinfection of ponds, continuous removal of moribund and dead animals, destruction of all infected and exposed shrimp by incineration or burial.

Taura Syndrome: This is a serious problem for *P. vannamei* culture due to high level of mortality.

- Causative agents: Positive sense single stranded RNA virus called Taura syndrome virus.
- Transmission: Cannibalism of weak or moribund shrimps is most efficient way of transmission followed by contaminated water source. Vertical transmission from from broodstock to offspring also exists.

- **Sign and symptoms: Pale reddish pigmentation and soft shells, multiple irregularly shaped and randomly distributed melanised cuticular lesion, death during ecdysis are major symptoms.**
- Diagnosis: Presence of dead or dying shrimp in cast net during routine sampling, hovering of predatory birds to diseased ponds, and cuticular melanized spots on moribund animals provide a strong presumptive diagnosis. TSV infected shrimp histopathologically display necrosis, and nuclear pyknosis of the cuticular epithelium of the general body surface, appendages, gills etc. The lesion is characterized by the presence of inclusion bodies that give TSV lesion a "peppered" or "buckshot" appearance which is considered to be pathognomonic for the disease. Disease can also be detected by In situ hybridization with specific DNA probes, RT-PCR, and antibody based ELISA.
- Prevention and control: Culture of TSV resistant species such as the western blue shrimp *Penaeus stylirostris*, stocking of specific pathogen free (SPF) or specific pathogen resistant (SPR) shrimp, high stocking density to reduce the impact, maintenance of optimal water quality, elimination or screening of potential carrier, destruction of all infected and exposed shrimp, strict isolation of outbreak pond, thorough cleaning and infection of infected pond.

Monodon Baculovirus (MBV): Monodon baculovirus is the first reported virus of *P. monodon* and the second virus of penaeid shrimp. MBV is a common, widespread pathogen in the shrimp hatchery and in acute cases mortality may go upto 70-90% of the stock. But, juvenile and adult *P. monodon* are comparatively resistant.

- Causative agent: Double stranded circular DNA virus of family Baculoviridae.
- Sign and symptoms: Infected shrimp shows lethargy, anorexia, dark coloration, and heavy surface fouling. Severely affected larvae and post larvae exhibit a white midgut line through the abdomen.
- Diagnosis: The target organs of MBV are hepatopancreas and anterior midgut. Disease can be diagnosed by histopathological examination of hepatopancreas which shows intranuclear inclusion bodies. DNA dot hybridization on fixed tissue section and Digoxigenin labeled DNA probes.
- Prevention and control: The prevalence of MBV in the hatchery can be substantially reduced by washing the eggs or nauplii before they are transferred to rearing tanks.

The best way to eliminate MBV from hatchery is to identify carrier broodstock or to spawn females individually and discard contaminated batches of larvae.

Infectious Hypodermal and Haematopoietic Necrosis (IHHN): The disease is caused by single stranded DNA virus of family parvoviridae. In *P. vannamei* it causes the chronic disease called runt deformity syndrome (RDS) characterized by lower overall crop production, shrimp with increased size variability, and cuticular deformity.

Hepatopancreatic Parvovirus (HPV): Hepatopancreatic parvo virus is a single stranded DNA virus. High levels of HPV infection occurs in early juvenile stages result into slow growth which finally stops to grow at approximately 6 cm in length. Horizontal transmission occurs by cannibalism and vertical transmission by infected broodstock is also reported. Diagnosis is done by demonstration of single prominent basophilic intranuclear inclusion bodies in the hypertrophied hepatopancreatic epithelial cells.

Bacterial Disease of Shrimp

The bacteria causing diseases of penaeid shrimp constitute part of the natural microbial flora of seawater. Thus, bacterial infections of shrimp are primarily stress related. Adverse environmental conditions or mechanical injuries are important predisposing factors. The most common shrimp pathogenic bacteria belong to the genus *Vibrio*. Other Gram-negative bacteria such as *Aeromonas* spp., *Pseudomonas* spp., and *Flavobacterium* spp. are also occasionally implicated in shrimp diseases. Bacterial disease of shrimp is presented in table 1.

Table 1. Bacterial disease of shrimp

Sl. No.	Disease	Causative agents	Sign and symtom	Prevention & control
1	Systemic Vibriosis	*Vibrio spp.*	Lethargic, reddish coloration on pleopods, gill eroded (German hamlet), black blisters on carapace and abdomen.	Good water quality, avoiding overcrowding, Oxytetracycline fortified feed.
2	Luminescent Bacterial Disease	Luminescent bacteria *Vibrio harveyi*	Affected larvae shows green bioluminescense under darkness, high mortality in hatchery.	Daily exchange of water in the hatchery, disinfect hatchery facility.
3	Vibriosis in larvae	*V. alginolyticus, V. parahemolyticus, V. Angullarum*	Necrosis of appendages in affected larvae, expanded chromatophore, empty	Good water quality, reducing organic load.

			gut, absence of fecal strand.	
4	Brown spot disease or Shell disease	*Vibrio spp. Aeromonas spp., Flavobacterium spp* with chitinolytic activity	Brownish to black eroded area on body surface.	Induction of moulting by tea seed, water exchange to reduce organic load, reduce overcrowding and unnecessary handling.
5	Necrosis of appendages	*Vibrio, Pseudomonas, Aeromonas Flavobacterium*	Tips of walking legs, swimmerets and uropods gets necrosed and become brownish and black, antennae and appendages may be broken.	Induction of moulting by applying 0.5 – 1 ppm tea seed cake, maintain good water quality and optimum density of shrimp.

Fungal, Parasitic and Toxic diseases of shrimp

Apart from virus and bacteria, other pathogens such as fungus and parasites are responsible for causing important diseases such as larval mycosis, protozoan fouling etc, in shrimp. Further, pond toxic environment are also responsible for causing some important diseases such as loose shell syndrome, black gill disease etc. The list of all such diseases has been tabulated in Table 2.

Table2. Fungal, Parasitic and Toxic diseases of Shrimp

Sl. No.	Disease	Causative agents	Sign and symtom	Prevention & control
1	Larval mycoses	Fungi like *Lagenidinium, Sirolpidium*	Larvae appear opaque followed by sudden mortality, protozoea and mysis stage are highly susceptible, within 1-2 days of larval stock may die	Remove bottom sediments and dead larvae periodically, disinfect hatchery facility, Treflan 0.1 -0.2 ppm bath for 1 day.
2	Protozoan Fouling	Peritrichous flagellates like *Zoothamnium, Epistylis, Vorticella* etc. (Protozoan diasease)	Restlessness, respiratory and locomotory function impaired, fuzzy mat appearance on body surface in highly infected shrimp.	Maintain good water quality, reduce organic load, silt and sediment. Treatment with formalin 15-25 ppm for pond or dip treatment of affected animals in 50-100 ppm

				for 30 min. is very effective.
3	Cotton shrimp disease or Milk shrimp disease	Microsporeans such as *Agmasoma*, *Ameson* and *Pleistophora* (Protozoan diasease)	Muscle appear cooked, exoskeleton appear bluish black. whit tumour like swelling on gills and subcuticle.	Affected animals should be destroyed and buried away from the farm.
4	Soft Shell Syndrome	Non infectious Sudden fluctuation of water quality, high soil pH, Highly reducing condition in soil, low organic and phosphate content, pesticide pollution.	Cuticle loose, soft and papery, Undulating gut, Poor escape reflex.	Good water and sil condition inpond and provision for balanced diet.
5	Black gill disease	Excess level of ammonia, nitrite, heavy metals, crude oil etc in culture pond. Bacterial, fungal and protozoans are also involved.	Brown to black discoloration of gills, necrosis and atrophy of gill lamellae.	Good water quality should be maintained with less organic content.

Conclusion

In last twenty years many lethal viral pathogens like WSSV, Taura syndrome virus etc. were discovered in shrimp culture and its pathogenesis and prophylaxis has been elucidated in detail. However, similar interest in diseases for brackishwater finfish culture is lacking probably due to less incidence of disease occurrence in finfish culture compared to shrimp sector. Still, few finfish diseases like viral nervous necrosis, iridovirus, ulcerative epizzotic syndrome are now fetching interest due to some worst mortality reported.

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MONITORING OF MICROBIAL STATUS IN FISH AND SHRIMP CULTURE

R. Ananda Raja, Akshaya Panigrahi, Debasis De and Sujeet Kumar

Introduction

The shift from extensive to semi-intensive and intensive farming has brought about an increase in disease outbreaks, especially by bacteria. It is essential to enumerate and identify the kinds of microbiota in the sample to know either beneficial or deleterious role in fish and shrimp culture. Microbes are extremely versatile and can show considerable morphological and/or physiological variation. Hundreds of thousands of microbial species have been defined, each with its own particular characteristics and requirements. It involves enormous experience and knowledge to isolate and characterize the particular bacteria responsible for disease by proving the Koch's postulate. So, it is not possible to explain all in one chapter. This chapter is aimed at explaining the methodology and the importance of total viable plate count (TPC) and total vibrio count (TVC) in fish and shrimp culture system.

Sterilization

As under normal circumstances bacteria are present in almost all situations exposed to the atmosphere, it is desirable to eliminate the existing bacterial flora from unsterilized media and the surfaces of the glassware. The sterilized media and other materials are prepared and kept sterile throughout their use. Sterilization can be accomplished by physical (dry heat, moist heat and filtration) or chemical (antiseptics and disinfectants) means. The most commonly used heat sterilization is described here under.

i) Sterilization by dry heat

Certain materials like inoculation loop, spatula, mouth of test tubes, etc., can be sterilized directly on the flame of the Bunsen burner.

a) Hot air oven

The materials which can be sterilized by hot air oven are glassware, all glass syringes, powders, fats and oils. Before sterilization, glassware must be dry and properly wrapped in papers. Serological pipettes may be sterilized in metal cases. Flasks and test tubes should be properly stoppered with non-absorbent cotton wool. It is customary to apply a temperature of 160° C to 180° C for at least one hour.

ii) Sterilization by moist heat

a) Serum inspissator

It is a fractional sterilization by moist heat at lower temperature. In this method, a temperature of 80° C for 2 hours on three successive days is maintained and the medium should be incubated at 37° C in between the sterilization timings.

b) Tyndallization / Arnold sterilizer

The temperature higher than 100° C will affect certain media and solutions (e.g., sugar media) adversely. Sterilization at 100° C can be accomplished in Arnold sterilizer with free flowing steam. Since heat will not destroy all spores at 100° C, the method of fractional sterilization (tyndallization) must be used for complete sterilization. This consists of heating the media to a temperature of 100° C for approximately 30 minutes for 3 successive days. This process permits the resistant spores to germinate during the intermittent incubation periods between two successive sterilizations.

c) Instrument sterilizer

Certain instruments like forceps, scissors, syringes, needles, etc., can be sterilized by boiling them in water for 15 minutes.

d) Autoclave

The most effective and reliable method of sterilization is by steam under pressure which is accomplished by an autoclave. Moist heat denatures protein at a lower temperature than dry heat. It is usually sufficient to expose the material at the temperature of 121° C for 15 minutes at 15 lbs pressure. This will kill all bacteria and their spores. Most of the bacteriological media, aprons, bandages, surgical instruments, syringes, rubber appliances, discarded cultures, etc., are safely and effectively sterilized by this method.

Preparation of media

The composition of the important mediums primarily used for enumeration, isolation and identification of bacterial colonies from the sample of interest.

Tryptone Soya Agar (TSA) – Composition (Gms/L)

.Pancreatic digest of casein	-	15.00
Papaic digest of soyabean meal	-	05.00
Sodium chloride	-	05.00
Agar	-	15.00

Make the volume up to 1000 ml using distilled water and the final pH at 25° C is adjusted to 7.3±0.2. Autoclave at 121° C for 15 minutes at 15 lbs pressure.

Zobell Marine Agar - Composition (Gms/L)

Peptic digest of animal tissue	-	05.00
Yeast extract	-	01.00
Ferric citrate	-	00.10
Sodium chloride	-	19.45
Magnesium chloride	-	08.80
Sodium sulphate	-	03.24
Calcium chloride	-	01.80
Potassium chloride	-	00.55
Sodium bicarbonate	-	00.16
Potassium bromide	-	00.08
Strontium chloride	-	00.034
Boric acid	-	00.022
Sodium silicate	-	00.004
Sodium fluorate	-	00.0024
Ammonium nitrate	-	00.0016
Disodium phosphate	-	00.008
Agar	-	15.00

Make the volume up to 1000 ml using distilled water and the final pH at 25° C is adjusted to 7.6±0.2. Autoclave at 121° C for 15 minutes at 15 lbs pressure.

Nutrient Agar – Composition (Gms/L)

Peptic digest of animal tissue	-	05.00
Beef extract	-	01.50
Yeast extract	-	01.50
Sodium chloride	-	05.00
Agar	-	15.00

Make the volume up to 1000 ml using distilled water and the final pH at 25° C is adjusted to 7.4±0.2. Autoclave at 121° C for 15 minutes at 15 lbs pressure.

Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar – Composition (Gms/L)

Proteose peptone	-	10.00
Yeast extract	-	05.00
Sodium thiosulphate	-	10.00
Sodium citrate	-	10.00
Oxgall	-	08.00

Sucrose	-	20.00
Sodium chloride	-	10.00
Ferric citrate	-	01.00
Bromo thymol blue	-	00.04
Thymol blue	-	00.04
Agar	-	15.00

Make the volume up to 1000 ml using distilled water and the final pH at 25° C is adjusted to 8.6±0.2. Do not autoclave.

Collection of water, fish and shrimp sample

Good laboratory technique is essential particularly in microbiological laboratory procedures. Care in sample collection and preservation, a clean laboratory and work surface, proper sterilization and inoculation practices, and close temperature control assure reliable results. Proper sampling procedures will insure that the results are representative of the sample source. Using a sterile container, 100 ml of water sample should be collected keeping at least 2.5 cm of air space to allow adequate space for mixing the sample prior to analysis as per the standard methods. Care must be taken to avoid sample contamination during collection. No dechlorination is necessary if the sample is added directly to the medium on site. Otherwise, samples should be treated to destroy chlorine residual and immediately transported for analysis after collection. Sodium thiosulfate, sterilized within the collection container, is commonly used to destroy chlorine residual. Allow no more than 6 hours to elapse between collection and examination for nonpotable water samples and 30 hours for potable water samples. For best results, the sample should be inoculated within 6 hours after collection and should be maintained at or below 10 °C, but not frozen.

Aseptically 1 g of the fish or shrimp sample under study is collected and homogenized with 9 ml of normal saline (10^{-1} dilution) and then serially diluted as per the need.

Pour plating

The pour plate method requires use of 1 ml, 0.1 ml, and 0.01 ml or 0.001 ml of sample. The difficulty measuring and working with the two smaller volumes, 0.01 and 0.001 ml, require the use of sample dilutions. These dilutions are prepared by pipetting 1 ml of undiluted sample into 9 ml of buffered dilution water. Diluting the sample allows 1ml of diluted sample to be used instead of 0.01 ml of undiluted sample, and 0.1 ml of diluted sample instead of 0.001 ml of undiluted sample. The TSA medium is preferable for counting total heterotrophic bacterial population and 15-18 ml of the medium is poured to each

dilution and mixed well and allowed to set with inoculums. After solidification, the plates are incubated upside-down at 35° C for 48 hours.

Spread Plating

The sterile TSA plates are inoculated and spread uniformly with 200µl of 10^{-1} to 10^{-10} dilutions of samples in duplicate and the plates are incubated upside-down at 35° C for 48 hours. Between spreading operations of each plate, the glass rod used for spreading is sterilized by dipping in alcohol and flaming.

After 48 hours of incubation, the colonies developing in the each plate are counted using Quebec colony counter. The sample volumes and dilutions are selected to get the total number of colonies on a plate between 30 and 300. The number of samples to be plated at any one time is limited so that not more than 20 minutes (preferable 10 minutes) elapse between the dilution of the first sample and the pouring of the last plate. The sample container is mixed thoroughly before performing dilutions (approximately 25 times) and sterile pipette is used with sterile microtips for each sample. TPC i.e. Colony-Forming Units (CFU)/g of sample is calculated as follows.

CFU/g sample = Average count x Dilution factor.

In the case of colony counts from spreaders, the average count has to be doubled before calculation of TPC. The spread plate technique is commonly used for TVC with the same procedure except using TCBS agar.

Significance of Total viable plate count and Vibrio count

1. Antibiotics have been used indiscriminately to control these diseases. To avoid the use of antibiotics and the development of resistant strains of bacteria, monitoring and maneuvering the TPC and TVC in culture systems and hatcheries are important.
2. TPC and TVC are used as indicators to monitor the alarming health status of the culture.
3. They are used further to avoid microbiological hazards from harvested products.

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APPLICATION OF POLYMERASE CHAIN REACTION (PCR) IN AQUACULTURE

R. Ananda Raja and Sujeet Kumar

Introduction

PCR refers to Polymerase Chain Reaction, a technique widely used in molecular biology. It was developed in 1983 by **Kary Mullis** and has become indispensable technique used in medical and biological research. In 1993, **Kary Mullis** won the **Nobel Prize** in Chemistry for his work on PCR. PCR derives its name from one of its key components, a DNA polymerase used to amplify a piece of DNA by *in vitro* enzymatic replication. As PCR progresses, the DNA thus generated is itself used as template for replication which is further exponentially amplified.

Components and reagents

1. Autoclaved Distilled Water: To make up the desired volume for the reaction.

2. Buffer solution: It provides a suitable chemical environment for optimum activity and stability of the DNA polymerase.

3. Mg^{2+} concentration: Since Mg^{2+} ions form complexes with dNTPs, primers and DNA templates, the optimal concentration of $MgCl_2$ has to be selected for each reaction. Too few Mg^{2+} ions result in a low yield of PCR product, and too many increase the yield of non-specific products and promote misincorporation. Lower Mg^{2+} concentrations are desirable when fidelity of DNA synthesis is critical. Standard Mg^{2+} concentration is 2 mM, but sometimes it may need to be raised (rarely lowered) to get a PCR to work. Raising Mg^{2+} lowers specificity as happens in lowering the annealing temperature. It may cause multiple bands to appear. But, usually the concentration of $MgCl_2$ should be selected empirically, starting from 1 mM and increasing in 0.1 mM steps, until a sufficient yield of PCR product is obtained. If the DNA samples contain EDTA or other chelators, the $MgCl_2$ concentration in the reaction mixture should be raised proportionally.

4. dNTPs: The concentration of each dNTP in the reaction mixture is usually 200 µM. It is very important to have equal concentrations of each dNTP (dATP, dCTP, dGTP, dTTP), as inaccuracy in the concentration of even a single dNTP dramatically increases the misincorporation level. When maximum fidelity of the PCR process is crucial, the final dNTP concentration should be 10-50 µM, since the fidelity of DNA synthesis is maximal in this concentration range.

5 & 6. Primers: PCR primers are usually 15-30 nucleotides in length. Longer primers provide higher specificity. The C and G nucleotides in primers should be 40 – 60% and

distributed uniformly throughout of the primer. More than three G or C nucleotides at the 3'-end of the primer should be avoided, as nonspecific priming may occur. The primer should not be self-complementary or complementary to any other primer in the reaction mixture, in order to avoid primer-dimer and hairpin formation. The melting temperature of flanking primers should not differ by more than 5°C, so the GC content and length must be chosen accordingly. All possible sites of complementarities between primers and the template DNA should be noted.

The melting temperature (Tm) is calculated using the following formula:

$$Tm = [\ (\text{Percentage of GC content in Primer X } 0.41) + 62.3] - \left[\frac{500}{\text{Primer length}} - 5\right]$$

The annealing temperature should be approximately 5°C lower than the melting temperature of primer.

7. DNA template: that contains the DNA region (target) to be amplified.

8. DNA Polymerase: In 1957, **Arthur Kornberg** identified the first DNA polymerase and was awarded the Nobel Prize in 1959 for the same. In 1969, **Thomas Brock** reported the isolation of a new species of thermophilic bacterium, *Thermus aquaticus* from which Taq DNA polymerase was isolated in 1976. The enzyme, Taq DNA polymerase is world widely used in PCR reaction. It should be stored at -20°C in a non-frost free freezer, typically in 50% glycerol. The tubes should never be allowed to reach room temperature and gloves should be worn when handling to avoid contamination. Before opening a new tube of enzyme, it is spun briefly as there is often enzyme in the cap. When pipetting enzyme from a stock tube, the end of the tip is just plunged far enough into the enzyme to get what is needed to avoid excessive adherence of enzyme to the peripheral tips. Enzyme should never be added to unbuffered water to avoid its denaturation. The volume of the enzyme should be less than $1/10^{th}$ of the final volume of the reaction mixture, as too much glycerol can interfere with enzyme activity. Usually 1-1.5 U of *Taq* DNA polymerase is used in 50 µl of reaction mix. Higher *Taq* DNA polymerase concentrations may cause synthesis of nonspecific products. However, if inhibitors are present in the reaction mix (e.g., if the template DNA used is not highly purified), higher amounts of *Taq* DNA polymerase (2-3 U) may be necessary to obtain a better yield of amplification products.

The Cycling Reaction

There are three major steps in a PCR, which are repeated for 30 or 40 cycles. This is done on an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time.

1. Initial Denaturation: The initial denaturation should be performed over an interval of 1-3 min at 95°C if the GC content is 50% or less. This interval may be extended up to 10 min for GC-rich templates. If the initial denaturation is no longer than 3 min at 95°C, *Taq* DNA polymerase can be added into the initial reaction mixture. If longer initial denaturation or a higher temperature is necessary, *Taq* DNA polymerase should be added only after the initial denaturation, as the stability of the enzyme dramatically decreases at temperatures over 95°C.

2. Denaturation: During the denaturation, the double strand melts open to single stranded DNA, all enzymatic reactions stop. The complete denaturation of the DNA template at the start of the PCR reaction is of key importance. Incomplete denaturation of DNA results in the inefficient utilization of template in the first amplification cycle and in a poor yield of PCR product. Usually denaturation for 0.5-2 min. at 94-95°C is sufficient, since the PCR product synthesized in the first amplification cycle is significantly shorter than the template DNA and is completely denatured under these conditions. If the amplified DNA has a very high GC content, denaturation time may be increased up to 3-4 minutes. Alternatively, additives such as glycerol (up to 10-15 vol.%), DMSO (up to 10%) or formamide (up to 5%) may be used to facilitate DNA denaturation. In the presence of such additives, the annealing temperature should be adjusted experimentally, since the melting temperature of the primer-template DNA duplex decreases significantly when these additives are used. The amount of enzyme in the reaction mix should be increased since DMSO and formamide, at the suggested concentrations, inhibit *Taq* DNA polymerase by approximately 50%. Alternatively, a common way to decrease the melting temperature of the PCR product is to substitute dGTP with 7-deaza-dGTP in the reaction mix.

3. Annealing: Usually the optimal annealing temperature is 5°C lower than the melting temperature of primer-template DNA duplex. Incubation for 0.5-2 min. is usually sufficient. However, if nonspecific PCR products are obtained in addition to the expected product, the annealing temperature should be optimized by increasing it stepwise by 1-2°C. The primers are jiggling around, caused by the Brownian motion and ionic bonds are constantly formed and broken between the single stranded primer and the single stranded template. The more stable bonds last a little bit longer (primers that fit exactly) and on that little piece of double stranded DNA (template and primer), the polymerase can attach and starts copying the

template. Once there are a few bases built in, the ionic bond is so strong between the template and the primer which does not break anymore.

4. Extension: This is the ideal working temperature for the polymerase. Usually the extending step is performed at 70-75°C. The rate of DNA synthesis by *Taq* DNA polymerase is highest at this temperature. Recommended extending time is 1 min. for the synthesis of PCR fragments up to 2 kb and may be further increased by 1 min. for each 1000 bp. Primers that are on positions with no exact match get loose again (because of the higher temperature) and don't give an extension of the fragment. The bases complementary to the template are coupled to the primer on the 3' side (the polymerase adds dNTP's from 5' to 3', reading the template from 3' to 5'). Because both strands are copied during PCR, there is an **exponential** increase of the number of copies of the gene. Suppose there is only one copy of the wanted gene before the cycling starts, after one cycle, there will be 2 copies, after two cycles, there will be 4 copies, and three cycles will result in 8 copies and so on. The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. If the initial quantity of template DNA is higher, 25-35 cycles are usually sufficient.

5. Final Extension: After the last cycle, the samples are usually incubated at 72°C for 5-15 min to fill-in the protruding ends of newly synthesized PCR products. Also, during this step, the terminal transferase activity of *Taq* DNA polymerase adds extra A nucleotides to the 3'-ends of PCR products.

Actual Procedure

For the total reaction volume of 25µl, the following components are added in respective volumes.

Availability	Requirement / Reaction
1. Buffer without $MgCl_2$ 10x	- 2.5µl
2. $MgCl_2$ (25mM)	- 1.5µl
3. d NTPs (2.5mM)	- 2.0µl
4. Primer F	- 1.0µl (10pM)
5. Primer R	- 1.0µl (10pM)
6. Template (50-100ug)	- 1.0µl
7. Autoclaved Distilled Water	- 15.0µl

The PCR tubes are placed in the thermal cycler at 95°C for 5 minutes to remove all the secondary structures from DNA and immediately in ice for 5 minutes (initial

denaturation). Then, 1.0µl (0.1-0.2U/µl) of the Taq Polymerase is added to the mixture and the thermal cycler is run as follows.

1. Denaturation - 94° C for 20 seconds.
2. Annealing - 55° C for 20 seconds.
3. Extension - 68° C for 90 seconds.
4. 35 cycles
5. Final Extension - 68° C for 10 minutes.
6. 22° C forever.
7. Program End.

As a rule-of-thumb, at its optimum temperature, the DNA polymerase will polymerize a thousand bases per minute. Stringency (decreasing the number of bands to avoid non-specific bands) can be increased by increasing the temperature and vice versa. $MgCl_2$ concentration is inversely correlated to the stringency. DMSO can also be added to reduce the stringency.

Application of PCR

1. Use of PCR augments many methods, such as generating hybridization probes for Southern or northern hybridization and DNA cloning, which require larger amounts of DNA, representing a specific DNA region. PCR supplies these techniques with high amounts of pure DNA, enabling analysis of DNA samples even from very small amounts of starting material.
2. DNA sequencing.
3. Recombinant DNA technologies involving the insertion of a DNA sequence into a plasmid or the genetic material of another organism.
4. Different PCR-based methods are used in genetic fingerprinting to identify the extremely small amounts of target of interest.
5. Used to identify genetic relationships between individuals, such as parent-child or between siblings, and are used in paternity testing.
6. This technique may also be used to determine evolutionary relationships among organisms.
7. PCR may also be used in the analysis of ancient DNA that is thousands of years old.
8. A diagnostic application in pathology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific sequence.

Conclusion

PCR has gained its importance in modern molecular biology over a period of last two decades. So, it has become inevitable for any molecular biologist to understand the basics of PCR. The same is extensively used in diagnosis of various diseases, developing ds RNA therapy & vaccines to shrimp and fish diseases, developing transgenic fishes, and so on.

Suggested Readings:

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BROODSTOCK NUTRITION FOR SHRIMP AND FISH

Debasis De and T.K.Ghoshal

As the production of marine cultured fish increases, the demand for good quality seed has been continuosly arising. To satisfy that increasing demand spawning quality and seed production success must be improved by controlling the nutritional quality of broodstock diets and first-feeding regimes. Gonadal development, maturation, spawning, viability of gametes, fertilization rate, egg quality, hatachability rate, survivability of larvae, and fecundity in fish are greatly affected by broodstock nutrition in several fish species During the last decade, increasing attention has been paid to the role of different components of broodstock diets such as protein, essential fatty acids, vitamin E, vitamin C, carotenoids and phosphoglycerides. Development of technological package for captive broodstock is essential for reliable supply of spawner for artificial propagation. For this broodstock should be supplied with nutritionally adequate feeds and should be reared in stress free environment. Few nutritional study carried out in carp, rainbow trout, red sea bream, tilapia, seabass, and penaeid shrimp, revealed that the level and quality of protein, lipids, essential fatty acids, vitamins (A,D,E,C), minerals (Phosphorous), and carotenoids affect the performance of broodstock. Nutritional, physiological, biochemical and general biological information of candiadate species is required to design artificial diet. But, to date only limited information is available for broodstock of fish and crustaceans species. In spite of this, several empirical feed formulations are in use, which are either improved version of grow out diets for the same species or related species.

Nutrient requirements

Total lipids

Provision of lipid percent in shrimp feed is based upon the satisfaction of requirements for specific nutrients such as highly unsaturated fatty acids (HUFA), phospholipids and sterols, and for energy. Crustaceans have long been recognized as having limited ability to synthesize HUFA de novo and no ability to synthesize sterols de novo. The concentration of total lipid in artificial broodstock diets averages 10%. This is approximately 3% higher than in grow-out diets for shrimp. Some broodstock diets contain lipid levels of 14% or higher. However, very high dietary lipid levels may affect the ingestion rate in a negative way, given the fact that shrimp get satiated when their energy requirements are met. This may finally result in nutrient deficiencies. Reproduction trial with *Litopenaeus stylirostris* fed three different total lipid levels: 7.8%, 11.1% and 13.9% revealed that Nauplii production and zoea length were higher in the dietary treatment providing 11.1% total lipids.

It should be noted that a feeding regime of 40% squid and 60% dry diet was used, and that the 11.1% lipid level was obtained with an artificial diet that had 10.1% total lipids. In other words, at the time of formulating artificial diets, one has to keep in mind with which other diets it will be combined and in what proportions.A recent study demonstrated that total dietary lipid levels above 9% retarded ovarian maturation of *L. vannamei* spawners. Earlier work on lipid metabolism provides evidence for the transfer of lipids from the hepatopancreas to the ovary via the haemolymph during ovarian maturation. In all Penaeid species, an increase of total lipid concentration in the ovaries takes place, and in most species a concomitant decrease of the total lipid level in the hepatopancreas can be observed. It is therefore thought that the hepatopancreas is at the origin of the lipids accumulated in the ovaries. However, an increasing amount of evidence indicates that a major part of the accumulated ovarian lipids originate from the diet.

Fatty acids

One of the nutritional factors that have been found to greatly affect spawning quality in fish has been the dietary essential fatty acids content. It was observed in gilthead sea bream broodstock fed with diets containing up to 1% n-3 HUFA (highly unsaturated fatty acids)that the composition of the female organs which are associated with reproduction were modified by the essential fatty acid levels of the diet and could effect egg quality over a short time period. Dietary levels of EPA and Arachidonic acid have also been found to correlate with fertilization rate in gilthead sea bream broodstock. The ovarian lipids contain higher proportions of *n* - 3 HUFA, particularly 20:5n - 3 and 22:6*n* - 3, than those of the hepatopancreas, for which it is believed that they play a crucial role in shrimp reproduction. The importance of *n* - 3 HUFA has also been deduced from their presence in natural food organisms that are successful maturation diets, e.g. squid and bloodworm. Retarded ovarian development was found in *M. japonicus* fed a HUFA-free diet.

Table 1. Fatty acid composition (% of total fatty acids) of fresh food items and artificial diets used for shrimp broodstock.

Fatty acid	Fresh food		Artificial diet			
	Bloodworm (%)	Clam (%)	Mussel (%)	Experimental[51] (%)		Commercial (%)
16:00	7.5	25.0	13.6	20.7	19.4	29.2
16:1	3.6	5.7	6.1	7.2	3.5	4.6
18:00	6.5	6.8	3.0	4.3	3.3	4.9
18:1	7.1	15.4	4.2	16.5	15.6	15.6
20:4*n* - 6	4.1	2.4	2.7	0.8	1.1	0.3
20:5*n* - 3 (EPA)	29.3	18.1	15.3	8.4	10.0	4.0
22:6*n* - 3 (DHA)	12.9	6.8	17.5	6.7	19.8	13.2
n - 3 HUFA	52.0	24.9	33.9	15.1	31.2	17.8

Positive correlations were found between egg 20:5n - 3 levels and fecundity and between egg 22:6 *n* - 3 levels and hatching percentage. It is postulated that 20:5n - 3 plays a specific role in the ovarian development process, whereas 22:6*n* - 3 may play some other role in early embryogenesis. Still, the data presented in Table 1 seem to indicate that artificial diets have relatively low concentrations of arachidonic acid (20:4*n* - 6). 20:4*n* - 6 , a HUFA thought to be a precursor in the synthesis of prostaglandins, which may play a role in reproduction. A delicate balance exists between *n* - 3 and *n* - 6 fatty acids, and maturation diets should contain high *n* - 3 / *n* - 6 ratios. It was found that apart from high 20:5n - 3 and 22:6*n* - 3 levels, moderate levels of arachidonic acid (20:4 *n* - 6) should be included into the diet. Different studies determined a *n* - 3 to *n* - 6 ratio of approximately 2 to 1 in the mature ovaries of *P. semisulcatus* and *L. vannamei* spawners, respectively, while in nauplii of *L. vannamei* this ratio increased to 3 to 1.

Essential fatty acids, such as *n* - 3 and *n* - 6 HUFA play a very important role in marine broodstock nutrition, as unlike fresh water fish, marine fish are incapable of elongating the shorter chain fatty acids. Studies on gilthead sea bream have demonstrated that *n* - 3 HUFA are essential for good egg quality, indicating the importance of maintaining the balance between *n* - 3 HUFA and other shorter chain fatty acids for a high spawning quality. Even fatty acid profiles of semen have shown their dependence on dietary fatty acid profiles in yellowtail and the European sea bass and are likely to play an important role for optimal sperm motility and duration. The spawning quality was lower for crossings that were performed with sperm from males fed an *n* - 3 HUFA-deficient diet. This confirmed that the male spawning performance could also be affected by dietary composition.

Lipid classes

Biochemical studies on wild shrimp species demonstrated that phospholipids, triacylglycerides (TAG) and cholesterol are the main lipid classes in mature ovaries. During sexual maturation, a remarkable increase of TAG in the ovaries is observed in wild spawners. An increase in ovarian TAG from 1.09% to 39.65% in *P. semisulcatus* and 8.30% to 33.81% in *L. vannamei* ovaries, followed by a decrease to 20.6% in spent ovaries and a resultant level of 33.5% in nauplii was reported in different studies.

Phospholipids are predominant in shrimp ovaries, mainly phosphatidylcholine and phosphatidylethanolamine. Shrimp broodstock seem to have a dietary requirement for phospholipids. Improved nauplii production, hatching and spermatogenesis in *L. stylirostris* broodstock was observed by supplementing the diet with 1.5% soybean lecithin. Ovarian maturation in *M. japonicus* was retarded when their diet did not contain phospholipids.

Broodstock diet should contain more than 2% phospholipids in order to assure that 50% of the total egg lipids is represented by phospholipids, and for maintaining high spawn frequency and fecundity in *L. vannamei*.

Cholesterol is also an important lipid class in mature shrimp ovaries Cholesterol must be provided through the diet for growth of shrimp juveniles, and is assumed to be an essential dietary lipid for shrimp maturation and reproduction

Protein

It is assumed that protein requirements are higher during maturation and reproduction of animals as compared to the non-reproductive stages, given the intense biosynthesis that takes place during these processes. Artificial diets currently contain around 50% protein.

The optimal protein requirement of red sea bream is around 45%. In another study on European sea bass, reduced levels of dietary protein correlated with lower female body weight and low egg buoyancy and hatchability.

It is probably more important to address amino acids requirements, as 10 amino acids have been reported to be essential for crustaceans. Dietary amino acid profiles should be similar to those found in fresh food commonly used in maturation diets.

The protein contents of the hepatopancreas and the ovaries of the best performing animals were significantly higher. Females that did not spawn had the lowest protein levels in their tissues.

Carbohydrates

Carbohydrates are not essential for shrimp broodstock. However, they can be a useful inexpensive source of energy with protein-sparing and lipid-sparing effects. Complex sugars and polysaccharides are used more effectively than simple sugars. Starch is most commonly used. Carbohydrates are also excellent binders in diet formulation.

Carotenoids

Carotenoids are a group of pigments that cannot be biosynthesized by animals. They are taken up from the diet, and can be transformed afterwards from one carotenoid form into another. In juvenile shrimp, they are important for pigmentation. According to recent research, they do also play vital roles as natural anti-oxidant in shrimp larvae and broodstock. The effect of carotenoids on larval quality can probably be attributed to the anti-oxidant properties of carotenoids. Free radicals, initiated by various factors including active oxygen, attack lipids and proteins in biomembranes, leading to a deterioration of egg quality. Carotenoids, particularly astaxanthin, are strong scavengers of free radicals and protect eggs from oxidative deterioration. They also prevent peroxidation of poly-unsaturated fatty acids

(PUFA)in the diet. During early maturation, free and esterified carotenoids accumulate in the hepatopan-creas; during secondary vitellogenesis they are mobilized from there via the haemolymph to the ovaries. This accumulation of carotenoids in the ovaries during maturation results in their darkening, on which the "staging" of females into different maturation classes is based. The form of the carotenoids that accumulate depends largely on the diet. Free astaxanthin was predominant in maturing ovaries (up to 80% of the total carotenoids), increasing from 2 to 34 ppm. In the integument, carotenoid levels remained relatively constant (90 ppm) throughout the maturation cycle. A comparison with carotenoids of the natural diet of *P. esculentus* indicates that after ingestion dietary carotenoids are converted to astaxanthin. Studies on red sea bream also showed that fish fed frozen raw krill rich in astaxanthin had a high fecundity, but cuttlefish meal, cuttlefish meal oil and vitamin E also improved egg production. In the case of red sea bream, the diesterified astaxanthin component from krill meal was the determining factor for improved egg quality.

Vitamins

Fat-soluble vitamins A (or (β-carotene), D, and E were found to be essential to support shrimp growth. Dietary levels of thiamin, riboflavin, niacin, vitamin B_6, vitamin B_{12}, choline, inositol and ascorbic acid have also been recommended for maximal growth in several shrimp species. Nevertheless, the vitamin requirements for shrimp broodstock are yet to be defined, for which artificial broodstock diets are generally supplemented with a complete vitamin mixture.The vitamins that have been addressed up to date are A, C and E. Vitamin E has been shown to improve the percentage of normal sperm and the rate of ovarian maturation in *L. setiferus* after diet supplementation with 500 mg kg^{-1} toco-pheryl acetate. Reduced hatching percentage was observed in the low-vitamin E supplemented shrimp, which was correlated to a decrease of a-tocopherol levels in the egg from 400 mg g^{-1} dry matter (DM) to levels below 200 mg g^{-1} DM. The elevation of dietary a-tocopherol levels from 22 to 207 mg/kg significantly reduced the percentages of abnormal gilthead seabream eggs. Moreover, the elevation of a-tocopherol levels up to 127 % resulted in an improvement in fecundity, as expressed by the total number of eggs produced/female and egg viability, and as expressed as a percentage of normal eggs; the lowest fertility and larval survival rate reported in eggs from broodstock fed the lowest dietary levels of a-tocopherol. However, the a-tocopherol contents of eggs were not affected by an increase of dietary vitamin E levels up to 127 mg/kg, but further increases above this significantly increased the a-tocopherol level within the eggs. Egg quality in red sea bream was also found to be improved by the addition of vitamin E to broodstock diets. Ascorbic acid (vitamin C) levels in *F. indicus* eggs were

also affected by the dietary vitamin levels, and high hatch rate of *F. indicus* eggs was related to high ascorbic acid levels in the eggs.Ovarian maturation was retarded when diets were deficient in any one of the vitamins E, A and C.

Minerals

The mineral requirements of broodfish are not clearly established. The diet without supplemental phosphorous resulted lowest growth of Ayu broodstock with lower egg production. In red sea bream low in phosphorous produced large no. of abnormal eggs with two oil globules as against one oil globules in normal eggs. Mineral deficiencies or imbalances could affect crustacean reproduction negatively. Physiological stresses could trigger oocyte resorp-tion or reduce reproductive fitness of the broodstock. Additionally, mineral malnutrition could cause altered composition and quality of the eggs. There are, however, no publications on mineral requirements of shrimp broodstock, probably because of the following complications. Firstly, for minerals it is necessary to distinguish true dietary requirements and apparent physiological requirement, since the minerals can be absorbed from the water. Secondly, the input through animal meals in artificial diets is high, and performing purified diets that would allow controlled mineral levels do not exist.

Unknown maturation stimulating compounds

Shrimp maturation and reproduction are greatly influenced by environmental factors. In the wild, adult shrimp eat a wide variety of microinvertebrates (gastropods, bivalves, crustaceans and polychaetes) and plant material. In captivity, one tries to mimic breeding season conditions, in an attempt to trigger the hormonal machinery that controls maturation. Fresh or fresh-frozen marine organisms are used for acceptable maturation and reproduction outputs. Often, these marine organisms are found to give the best results when they are in a reproductive stage. Squid and bivalves (mussel, clam, oyster) are generally the main food items, fed at high daily ratios. Crustaceans like shrimp, crab and krill are also fed to shrimp spawners, but due to the risk of disease transmission, they are used less frequently nowadays. Bloodworms (marine polychaetes *Glycera dibranchiata* and *Americonuphis reseii*) and *Artemia* biomass are used for diet supplementation. Bloodworm is the most expensive ingredient used in hatcheries of the Western atmosphere, and maturation operators feel it to be indispensable for stimulation of ovarian maturation. *Artemia* biomass can also be included into artificial broodstock diets as a freeze-dried meal to increase diet ingestion and stimulate ovarian maturation. HUFA play a crucial role in shrimp reproduction, it should be remembered that lipid quality is not determined by its fatty acid composition only. Care should be taken not to overlook the contribution of nutrients other then HUFA. For example.

the high nutritional value of squid is also attributed to its amino acid composition—which is similar to that of shrimp and because it contains high sterol levels.

Artificial diets

According to a survey conducted among commercial hatcheries the development of artificial broodstock diets capable of replacing the fresh food is a priority. A lot of advantages can be expected from dry artificial diets over fresh food, e.g. reliable supply, reproducible and controlled quality, easy to use, improved stability under storage, reduced tank fouling, reduced risk of disease introduction and easy delivery of chemotherapeutics, immunostimulants, and/or hormones. Nevertheless, almost every attempt to completely replace fresh food with artificial diets, results in a decrease in ovarian maturation, a reduced number of spawns and an inferior egg quality. In most situations, a combination of fresh food and artificial diets gives better results than a feeding regime that consist of fresh food only.The commercial diets that are used are Breed S (INVE Aquaculture), Higashimaru (Higashimaru), MadMac-MS (Aquafauna Biomarine), Nippai (Japan), Rangen (Rangen) and Zeigler (Zeigler Bros.)

Conclusion

Still, our knowledge on the specific nutrient requirements of brackishwater shrimp and fish broodstock remains limited. Only purified or semi-purified artificial diets could provide the necessary tools to investigate the effect of certain nutrients on ovarian maturation, reproduction and offspring quality. This would be particularly useful for studying the requirements for dietary protein, amino acid profiles, energy:protein ratios and minerals, all of these being topics that remained unaddressed up to date. Even in the case of lipids, carotenoids and vitamins, questions remain as far as the quantitative requirements, nutrient interactions and nutrient metabolism are concerned. Biochemical studies can give further insight in metabolic pathways and provide useful tools to estimate nutrient requirements, to asses suitability of fresh food items for broodstock nutrition, and to determine egg quality and larval quality. Male reproducer nutrient requirements for optimal spermatophore development and high sperm quality should receive special attention, as most publications focused on females only. Finally, it must be remembered that broodstock maturation takes place over extended periods and the fish and shrimp must be cultivated under optimum conditions with minimum stress throughout this period. Thus there is a need for low stocking densities, good water quality, and appropriately formulated diets that are species specific and contain a selection of ingredients that improve fecundity and egg quality. The available information should be considered and applied to future studies that will lead to the formulation of wholesome

broodstock diets for currently cultured species. The development of dry diets that allow the complete replacement of fresh food is a research priority as well. Once our knowledge of the nutritional requirements and endocrinology of shrimp broodstock has improved, it will be possible to formulate artificial diets in such a way that a high, consistent and prolonged reproductive performance will be ensured without the need of eye-stalk ablation. The challenge will be to do it in a cost-effective manner at commercial scale.

Suggested Readings:

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USE OF PROBIOTICS IN AQUAFEED

Debasis De

The term "probiotics" was coined by Parker (1974) to describe "organisms and substances which contribute to intestinal microbial balance". Probiotics are living organisms which when introduced through host feed have a positive effect on host health. Some reside in the digestive tracts of the individuals while others have an external origin. They affect the host animal by improving its intestinal microbial balance (Fuller 1989). They are also sometimes referred to as 'Direct Fed Microbials (DFM)'. Probiotics can be used as growth promoters and also for therapeutic purposes. It can also be used to increase survivability and productions of shrimp in culture pond (Ghoshal *et al.* 2006) Probiotics include viable cultures of bacteria and fungi. In the gut, a variety of populations of microorganisms are present which are useful for the host organism. The population of gut microflora is affected by various factors namely, age, diet, environment, stress and medication. The most commonly used organisms in probiotic preparations are lactobacilli, streptococci and bifidobacteria. Except these, *Bacillus* spp., yeasts (*Saccharomyces* spp.) and filamentous fungi (*Aspergillus oryzae*) are also used as probiotics. The probiotic preparations are available as tablets, powders, capsules, pastes or sprays.

Properties of probiotics

- It should be resistant to pH and bile acids
- It should have the ability to attach to the gut epithelial lining.
- It should be non-pathogenic.
- It should provide a beneficiary effect to the host animal.
- It should possess a high viability.
- It should be stable on storage and in the field.
- It should survive the gut environment and should have the potentiality to colonize in gut.
- It should be cultivable on a large scale.

Probiotics generally find their applications in aquafeed because of their effects on high growth rate, improved feed conversion and improved resistance to diseases. The probiotic microorganisms in the gut stimulate the immune response of host system in two ways. They can migrate through the gut wall as viable cells thereby multiplying to a limited extent or the antigens which are being released by the dead organisms can be absorbed and stimulate the immune response directly. Some investigators believe that lactobacilli act

indirectly through an effect on other components of the gut flora. It is a product of this change which induces the immune response. There are many probable reasons for improved immunity like increased activity of macrophages shown by enhanced ability to phagocytose organisms or carbon particles, increased production of systemic antibody e.g. IgM and interferon and increased effect of local antibody at mucosal surfaces such as gut wall. The effect of lactobacilli on the host immune system can be measured by estimating the levels of macrophage enzymes. Lactic acid bacteria have received priority as probiotics in fish feed (Hagi *et al.* 2004). Lactic acid bacteria when included in diet of Atlantic cod increased their survivility rate when they were challenged by pathogen *Vibrio anguillarum*. Lactic acid bacteria produce acetate and lactate which proves helpful for inhibiting the growth of several species of *Vibrio* (Vazquez *et al.* 2005). Use of probiotics influence the specific and non-specific immunity in many fish species like rainbow trout (Nikoskelainen *et al.* 2003; Panigrahi *et al.* 2005) and gilthead seabream (Salinas *et al.* 2005). Probiotics help in reducing the mortality of larval stages of different fishes and pathogen-challenged fishes and they provide the needed enzymes useful for digestion. But, effectiveness of these probiotics is adversely affected by harsh conditions of extrusion or pellet manufacturing. There are also many regulatory issues regarding the application of probiotics in aquafeed. Sometimes, variable results are also obtained than expected after use of probiotics in feed. This is attributed to the fact that different probiotics contain different microorganisms which may act differently under variable situations and also they have their own metabolic pathways which differ from the others. Moreover, growth phases of the animal, the type of dosing used and health status of animal have also an effect. So, many times, use of probiotics does not give expected results.

Suggested Readings:

Agarwal. N, D.N. Kamra, L.C.Chaudhary, I.Agarwal, A. Sahoo and N.N. Pathak. (2002) Microbial status and rumen enzyme profile of cross-bred calves fed on different microbial feed additives.

Lim Chhorn and Webster, Carl D. (2001) Nutrition and fish health. The Howorth Press, INC, New York.

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RECENT ADVANCES AND APPROACH FOR PENAEID SHRIMP HATCHERY MANAGEMENT

Akshaya Panigrahi

Introduction

Many years passed after the first captive spawning of penaied shrimp by Fujinaga (1934) and a spectrum of shrimp breeding programs have been attempted and seed production technology has been revolutionized. There are initially Japanese (green water)-style and Galveston-style of hatchery management which now converge with the best of both the system coupled with the locality specific modification to produce disease free healthy post larvae. India have approximately 1.55 lakh ha of brackish water area being utilized for Shrimp farming out of a total 1.2 million ha of potential area and produces nearly 1.13 lakh tones of cultured shrimp in 2004. To support this production now there are close to 300 hatcheries with production capacity of approximately 10 billion post larvae and requiring almost 2 lakh broodstock per year. The significant improvements in penaeid shrimp hatchery practices along with the standard operating procedures are elaborated here.

WSSV and Changing scenario

The importation of PL from other Asian countries and poor management of the broodstock, the hatcheries and also the farms led to the outbreak of White Spot Syndrome Virus (WSSV) in India during 1994. WSSV has continuously affected the shrimp hatcheries and farms, and the action plans to combat the disease has led to changing the standard operational procedure (SOPs) and many effective ways to keep the disease at bay. Now, a strict adherence to biosecurity measures and real time assessment of health status of the stock during the production cycle, emphasis on SPF broodstocks, and consideration of the Hazard Analysis Critical Control Point (HACCP) are becoming the yardstick of success in a hatchery.

Hatchery design and infrastructure

Hatchery design should comprise separate facilities for quarantine, maturation, spawning, hatching, larval and PL rearing, indoor and outdoor algal culture, hatching of *Artemia* and feed preparation as though each one of them can operate independently. Supporting infrastructure including water intake, processing and distribution system and laboratory with disease dagnostic facility, facility for packing and transport are to be well integrated with the hatchery design.

Facility maintenance, disinfection and hygienicity

The pumps, water filtration and distribution system, aeration system could become potentially major source of pathogen shelter and entry. So periodic inspection and remedial measures including disinfection of the pipelines with suitable disinfectants like chlorine (500 ppm), potassium permanganate (KMnO4, 20 ppm), formalin (200 ppm). Airline pipes should be fumigated with formalin and/or alcohol and air and intake water subjected to uv treatments. Tanks and hatchery equipments should be washed and disinfected at the end of every production cycle. Similarly, all the filters should be washed, disinfected and replaced time to time.

Water intake system and treatments

Water withdrawn from sub-sand abstraction points in sandy intertidal areas, installed as low as possible close to the limit of the low spring tides followed by sedimentation and filtration is taken for hatchery use. Inlet water treatment currently involves mechanical separation of the suspended particles by filtration, chlorination (10-20 ppm) and dechlorination, however, sometimes not enough for total elimination of pathogens (vibrio) and associated with chloramine formation. To overcome the shortcomings of chlorination, additional (or only) sand filtration, then microfiltration, followed by ozonation and/or UV irradiation may be adopted. UV irradiation must reach >30 000 mws/cm2 in the incoming water flow, while the ozone content in water must be more than 0.5 µg/ml for 10 min for effective disinfection from viruses (including WSSV), bacteria, fungi and protozoa. The use of activated carbon filters, the addition of ethylene diamine tetraacetic acid (EDTA) and temperature and salinity regulation should also be attended.

Activated carbon filter is advisable before use for maturation or larval rearing to ensure that no chlorine byproducts or other dissolved organics are in the water supplied.

Biosecurity measures

Various levels and strategies for biosecurity may be employed depending on the hatchery facility, the diseases of concern and the level of perceived risk. The SOPs followed for the hatchery management should have all consideration to implement biosecurity. The protocols for biosecurity are easier to implement through HACCP approach.

biosecurity programme for a shrimp hatchery should include the following elements:

- Recruiting disease-free and healthy shrimp stocks;
- Effective quarantine for all incoming stock;
- Screening the incoming stock for disease (i.e. through PCR or other immunodiagnostic technology);

• Treatment of all incoming water sources to eliminate pathogens;

• Sterilization and maintenance of clean equipment and materials;

• Personal hygiene measures including washing of hands, feet and clothing;

• Risk assessment and methods to address them:

• Development and use of SPF and SPR stocks:

• Maintenance of optimal environmental conditions within all phases of the facility;

• Application of immune enhancers and probiotics

HACCP approach

It is a preventive risk management system for most critical factors including viral diseases. Critical limits are set at critical control points (CCPs) in the system where controls must be applied to prevent, eliminate or reduce a hazard.

1. Standard Operational Practices

The SOPs should be based on responsible aquaculture management practices fulfilling the requirements like biosecurity and HACCP compliance, safe, improved selection, effective and minimal use of therapeutants, drugs, hormones and other chemicals, good nutrition, effective operation and health management; proper disposal of wastes

1.1. Maturation and spawning

Hatcheries depend on sourcing of gravid females to obtain nauplii, while many more buy them from the nauplii production centre. Thus broodstock utilization by the hatcheries has declined significantly in the last decade. Recent price of the gravid female is much lower than the high price of Rs 30-50,000/- per gravid female. The broostock selected should be healthy with lack of red coloration, lack of white spots, absence of external fouling, necrosis and with developed ovaries

Broodstock maintenance: Introduction of any brood stock into the production system must be followed with proper quarantine and screening for bacterial and subclinical viral infections (i.e. by PCR). Broodstock infected with serious untreatable diseases should be immediately destroyed and those negative for MBV and WSD should be taken for maturation. For effective quarantine, the broodstock should be taken from the bag and passed through a dip of povidone iodine solution (20 ppm), potassium permanganate (100 ppm) or formalin1 (50-100 ppm) for 30–60 seconds. Probiotics for controlling bacterial load, 0.1 ppm of copper control ($Cu SO_4$) for filamentous bacteria in gills or one hour aerated bath treatment with 30-50ppm formalin for epicommensals are suggested. The broodstock is kept in maturation unit for sometimes before performing eye-stalk ablation required for maturation. High quality feeds comprising live polychaete bloodworms (*Glycera* sp.) (10–12 percent/d), fresh squid (*Loligo*

sp.) (6–10 percent/d) and live but deshelled bivalve mollusks (clam etc at 4-8 percent/d) or frozen artemia and krill or maturation pellets constitute the maturation feed.

SPF broodstock: Apart from our research on selective breeding and genetic improvement of domesticated *P. monodon*, Indian Govt is collaborating with Moana Technologies (Hawaii), to develop our own domesticated stock at the nuclear breeding centres including one at Andaman and Nicobar island. Biosecurity aspects would be taken care of with all stages up to F2 and a targeted 60,000 broodstock can be produced to cater the high health seed requirements.

The development of SPF and or SPR lines of *P.monodon* should be regarded as a long-term investment. Again it is coupled with the difficulties of domestication in *P. monodon*. Also SPF status is not heritable. Offspring of SPF shrimp are not SPF unless they are produced and maintained at a biosecure SPF facility. Once they leave that facility, they can no longer be termed SPF and should instead be referred to as "High Health".

Spawning: Broodstock should be maintained, spawned and hatched individually to avoid cross contaminations and vertical transmission. Water recirculatory system to provide stable good quality water should be incorporated. Production of high quality, disease free eggs and nauplii should be ascertained by practicing proper spawning and hatching procedures. Gravid females with spermatophore are selected formalin treated (formalin 100 ppm -3min dip) and taken for spawning. The eggs spawned per female should be in the range of 200 000–400 000 eggs for females of 90–150 g body weight, and up to 450 000–1 000 000 eggs for 160–300 g females. The fertilization rate is found out by observing under microscope within two hours and if below 50 %, the batch may be discarded. A suitable receptacle for harvesting the eggs, excluding broodstock faeces and ovarian tissues (using a prefilter made from 300–500 μm mesh, for example) is used and eggs are disinfected and transferred to hatching tanks.Testing of the spawned female for WSSV and MBV is done before using it for subsequent spawning.

1.2. Hatching

Hatching tanks are usually 200-500 L for individual and bigger tanks for communal hatching with conical bottom to facilitate water circulation, aeration and harvest. The stocking density for hatching is 1000 eggs/L and EDTA (10–30 ppm) and Treflan (0.05 –0.1 ppm) are usually added to the water as that in the spawning tank. After 12-15 h , nauplii (stage III/IV) are collected using light as they show positive phototaxic nature. The unhatched eggs and weaker nauplii are discarded after chlorination.

Health management: The principle of zero tolerance to antibiotics is the best option for a sustainable hatchery system. The allowed chemicals in appropriate dose handled by qualified

technician should only be acceptable. Environmentally friendly interventions might be equally effective. The eggs after cleaning in sea water should be dipped into an aerated bath of 50 ppm povidone-iodine solution for 1 min followed by 5-10min wash in slow and steady flow of clean sea water. The healthy nauplii after collection is washed in clean sea water followed by 100–300 ppm formalin for 30 seconds and dip into an aerated bath of 50–100 ppm povidone-iodine solution for 1 min and clean sea water wash. All lots of nauplii should be tested for WSSV by PCR before transfer to the larval-rearing tanks.

The healthy nauplii are transferred to the larval rearing tanks in plastic bags inside bucket and in case it is to be shifted far. 30,000 nauplii/ L density in oxygen pack bags can be sent in insulated polystyrene foam boxes to maintain temperature and reduce stress.

1.3. Larval rearing

Post spawning procedures include one of the important phase larval rearing management comprising LRT tank preparation, health management, nutrition and feed management, quality testing and post larval packing and transport followed by stocking in nursery/ grow-out facility.

The objective here is to produce high health post larvae which will have high growth and survival performances. The larval rearing sometimes comprises two phases i. e. first phase from nauplius up to PL4-5 and second phase up to harvest (PL 15-20).

Factors affecting larval quality and health include

- Optimum stocking density (75-150 nauplii/L)
- Minimum bacterial load
- Appropriate feeding programme (quality and frequency)
- Algal quality (Chaetoceros @80-130,000 cells/ml)
- Artemia nauplii (good quality)
- Water quality management (proper water management schedule)
- Minimal chemical and antibiotic use
- Probiotic use -beneficial

Larval-rearing tanks should be filled to only 50 percent of their full capacity with clean, disinfected, filtered seawater prior to stocking with nauplii and through the zoeal stage gradually the tanks are filled up and from mysis stage onwards 10-30% water exchange is done which is increased to 30-50% during the PL1-4 stage gradually increasing up to 60-100 % during PL 13-18 stage. Improper water management sometimes lead to accumulation of

unionized (NH_3, optimum <0.1 ppm) ammonia and nitrite (NO_2, optimum <0.1 ppm) up to critical concentrations, resulting in sublethal toxicity and proliferation of *Vibrio* sp. bacteria.

Antibiotics and chemotherapeutics

The pharmacologically active substances banned for use in Indian aquaculture is declared through CAA notifications. Also CIBA and MPEDA like organizations discourage use of chemicals and propagate minimum/no use of antibiotics which have much negative impact including consumer rejection in the export market. Antibiotic should not be used prophylactically. However if antibiotic use is unavoidable, only approved antibiotics at appropriate level should be used responsibly. Antibiotic use will only treat the symptoms of the problem and not the underlying cause, which is invariably associated with poor environmental control. So cause of the problem must be attended before indiscriminate use of antibiotics. Appropriate guideline for withdrawal period is necessary to follow since the product is meant for consumption.

Larval condition and health assessment

Assessment of the larval health and general condition are carried out on regular basis. The diseases mostly encountered in hatchery includes Monodon baculo virus (MBV), White Spot Syndrome Virus (WSSV), Baculoviral midgut gland necrosis virus (BMNV) among the viral diseases, Vibriosis (bacterial) and larval mycosis (fungal) and other protozoan diseases. Vibriosis is the most commonly occurring bacterial disease with variety of clinical signs such as necrosis of appendages; exuvial entrapment; reddening of the pleopods, pereiopods and gills; cessation of feeding; white intestine; excessive fouling; luminescence in the water and larval bodies. Proper water quality, feeding and monitoring of health and well being and following strict biosecurity norms as described and use of probiotic and bioremedial agents keeps the chances of outbreak low. Health assessment in the hatchery at three levels helps producing high health larvae (Level I:Examination of health condition, deformity, feeding behavious, activity and stress tolerance; Level II: Bacterial load of the system and animal, microscopic observation; Level III: Screening through PCR and other high tech tools)

Probiotic based hatchery

Probiotic based hatchery managements are more and more accepted where good strain of probiotic bacteria or bioremediators are used to keep the pathogenic strain at bay. However, the efficacy of the probionts and dose/frequency standardizations at different larval stages concurrent to the immune system development with the understanding of their mode of action is required to be elaborated. Data collection and record keeping regarding day to day

operations, larval health, treatments/chemicals used, water quality and other relevant information are to be performed and monitored.

Discharge water treatment

Water quality parameters must be monitored in the discharge in order to comply with the general standards and to prevent polluting the environment surrounding the hatchery. All water discharged from the hatchery including water originating from the quarantine areas) should be held temporarily and treated with hypochlorite solution (>20 ppm active chlorine for >60 min or any other effective disinfectant and then well aerated (to dechlorinate) prior to discharge. The feedback from the corresponding grow out culture with regard to performance of any batch of seeds are important for a comprehensive understanding and improvement of hatchery practice. In addition to practicing improved hatchery techniques the hatchery must update itself and OSPs.

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OVERVIEW OF FARMING SYSTEMS WITH SPECIAL REFERENCE TO BIOSECURED ZERO WATER EXCHANGE SYSTEM TECHNOLOGY

A. Panigrahi and Shyne Anand

Introduction

Shrimp aquaculture has expanded rapidly, mainly in the subtropical and tropical lowlands of Asia and America and constitutes an important activity in coastal ecosystem. West Bengal is the highest fish producing state of India and in 2002-03, 11.20 lakhs Mt of fish were exported earning 533 Crores rupees. In this coastal terrain there is vast scope of shrimp farming either in monoculture or polyculture. However, worldwide shrimp farming activity is plagued by diseases and unsustainable practices sometimes affecting the coastal ecosystem and livelihood. Evolving culture practices are in place time to time to mitigate these concerns and increase production yield and sustainability. Traditional aquaculture includes management practices that have evolved through centuries to create agricultural systems adapted to local environmental and cultural conditions. Now with all the environmental and disease problems, the viability of commercial scale operation of traditional system is doubtful. With gradual increase in the management level and stocking density as well as production performances extensive, semi-intensive and intensive systems of farming are in practice. The zero water exchange shrimp farming system is an evolving culture practice which provides a means to achieve higher degree of biosecurity. This article describes the evolving culture practices in shrimp aquaculture as practiced by progressive farmers including that of the zero water exchange system of shrimp farming.

Background: Depending on the stocking density and level of management, conventionally the farming systems are classified as that of traditional, extensive, modified extensive, semi-intensive and intensive systems. While there are wide divergences in the manner with which these are accomplished, each has its common denomination which makes it a system apart.

Traditional system: Traditional culture with stocking 5- 25,000 PL/ha (mostly auto stocking) often in polyculture with fish, and average production not exceeding 500 kg/ha/yr depends completely on natural food and tidal flushing. This system relies mainly on the natural fertility of the soil and water and the amount of extraneous feed which the water brings along as it enters ponds. The size of the ponds is bigger sometimes range from 10 ha up to 100 ha.

Extensive system: generally involves smaller ponds with very little operational inputs. 30,000-100,000/ha (3-10/sq m), Supplemental wet or dry feeds are more or less regular in extensive culture, however, may be minimal, at best supplementary. In addition, fertilizers help stimulate a natural food chain.

Semi-intensive system involves a more systematic and scientific approach which involves the use of fertilizers to increase natural food and feeds to supply adequate nutrition. The use of these additional inputs paves the way for higher stocking rates (10-25/sq m). Partial water exchange is also necessary to keep conditions more or less within optimum and tolerable limits of the organisms.

Intensive system: This system is characterized by increasing stocking rates supported by corresponding higher feed and water management inputs. In intensive ponds stocking density is at 25-30/sq m or more, feeding and water management are completely dependent on formulated pellets, pumps and aerators. Most of the feed is consumed by the shrimp and less is available to serve as a stimulant to the natural food web. Average unit production is 0.6-1.5 mt, 2-6 mt and 7-18 mt/ha, respectively for extensive, semi- intensive and intensive culture; however this margin overlaps from region to region.

Super-intensive system: This system demands even greater control of the environment and stocked with still higher density produces 20-100 mt/ha or more. As densities increase, the farms get smaller, the technology gets more sophisticated, capital costs go up and production per unit space increases dramatically.

Evolving farming systems: These are modern farming systems with next level of modifications evolved based on various criteria and developing situations. Depending on the access of the system to outer water body; open farming system is modified to, semi-closed and closed farming system. To avoid pollution and disease from coastal water exchange, natural predators, weather peculiarities, and the side effects or long-term effects of antibiotics and other chemotherapeutics, Closed or Semi-closed systems have been evolved. Again based on species mix, alternation of crop, seasonality, fry stocking and harvesting, water source, stocking and harvesting, there is variation in farming systems.

Closed farming system: This farming system operates in a more environmentally friendly fashion and attempts to overcome problems inherent in the "open production systems.". Biosecurity issues can also be adequately addressed in such a system. Recycling of the effluent waters emanating from the production ponds can be done by complex and costly water filtration systems to establishment of settlement ponds, or integrated secondary

containment ponds. Though the initial financial risk is steep, the closed-system eliminates many of the production risks.

Semi-closed farming system: These are the integrated system which essentially incorporates a biopond where oysters and other shell fish, fin fish, and seaweeds are being cultivated either together with the shrimp, or in separate but interconnected ponds. These ponds provide many nutrients for the other cultured species, which in turn can filter out a lot of the particulate matter and pollutants, thus helping to purify the fouled waters.

Organic farming system: The eco-cultural principles, which traditional methods are based on, can be successfully adapted to or modified following organic principles as defined by IFAOM, Naturland and other international certification agency. At CIBA, we have demonstrated the viability and associated economic and environmental advantage of these low input, low stocking systems following organic principles, though certification process is yet to be addressed. Other than these systems, depending on the integration type Paddy-shrimp, Livestock-shrimp farming systems are also prevalent though not very common.

Zero water exchange farming system: In light of the devastating disease problems currently plague the global shrimp farming industry, water exchange thus become a risky management option High quality disease free shrimp stocks and quality feeds are utilized, there is evidence that water exchange can be reduced, perhaps to zero, in many instances. The Biosecured Zero Water Exchange System Technology (BZEST) can be applied to extensive and semi-intensive/ intensive shrimp aquaculture.This system mostly relies on zero or minimal water exchange where monsoon precipitation taking care of the evaporation loss.

The Principle: Unlike the open system where water replacement is done as per the level of intensification the zero water exchange shrimp farming system is a closed system which provides a means to achieve higher degree of biosecurity. This biosecured system ensures the prevention of bacterial/viral contamination which is the major bottleneck to have sustainable shrimp farming. This technology can be applied to extensive and semi-intensive/ intensive shrimp aquaculture. Once the disinfected (with 60 ppm of chlorine) water is taken and cultured for optimum bloom, no further water is taken from the source and the evaporation loss etc. is compensated by treated water or the crop is so scheduled to take the advantage of rainwater for that purpose.

Uniqueness of BZEST and biosecurity: Biosecurity is the protection of living organisms by the Exclusion of Pathogens and Other Undesirables. Biosecurity is relatively new to aquaculture, the adoption of biosecurity protocols in shrimp aquaculture has resulted in the shrimp overall production increase. Success of establishment and implementation of

biosecurity program in shrimp aquaculture system demands the pivotal role of cluster farming in which each individual farmer plays crucial role. Thus Zero water exchange system provides a means to achieve higher degree of biosecurity in shrimp culture system.

BZEST and biotherapeutic agents: *In situ* microorganisms help in regulating biogeochemical cycles within the culture environment and in directly affecting shrimp growth and survival Effective recycling of nutrients and other metabolites and maintaining a stable environmental quality through the use of probiotics or bioremediations is believed to be the driving force for use of such biotherapeutic agents. The application of a group of beneficial microorganisms (such as *Lactobacillus, Bacillus, Nitrosomonas, Cellulomonas, Nitrobacter, Pseudomonas*) is reported to be very useful for controlling the pathogenic microorganisms and water quality. Positive microbial activities include elimination of toxic materials such as ammonia, nitrite, and hydrogen sulfide, degradation of uneaten feed, and nutrition of aquatic animals such as shrimp, fish influencing production.

BZEST and the environment: The gross primary production, phosphate and nitrate level is maintained at higher level in the BZEST system. However, sometimes unregulated feeding may lead to eutrophication and algal bloom. The nitrogenous metabolite like ammonia, nitrite should be low. Other parameters influencing productivity like alkalinity, pH, and dissolved oxygen similar to other conventional culture. The organic matter in the ponds after the crop is sometimes high; requiring sludge removal and soil scrapping at the end of the culture.

BZEST and disease outbreaks: This system by means of all biosecurity measures defenses against rampaging protozoa, fungi, bacteria, and viral diseases that pose the greatest threat for shrimp aquaculture. Again, use of beneficial microbes reduces the chance of any disease occurrences. Though there is no remedy to treat shrimp viruses, but management techniques including this have evolved which can lessen the chances. However, since this closed system of farming is new, the possibility of any emerging diseases (like slow growth syndrome, white fecal disease and bacterial and protozoan diseases) should be thoroughly looked in to. A very strict feeding regime associated with the biotherapeutic use improves the system's defense against any possible disease outbreak.

Other Factors for assured production:

- Healthy hatchery (SPF or high health stock) seeds for stocking
- Biosecurity measures
- Quality Feed and strict feeding regime: As farms evolve from low to high stocking densities, the quality of feed becomes very important. The detritus food web develops

in this system induced bacterial flocs, so the protein levels in the feeds may be reduced.

- Minimal water requirements: water is added during the production cycle only to replace seepage and evaporation losses and the crop is so scheduled to take the advantage of rainwater for that purpose.
- Aeration: Heavy aeration (through Paddlewheel or aspirating aerators) always helps in such closed system
- Microorganisms (Autochthonous & Allochthonous) and associated detritus as a source of supplemental food for the shrimp
- Stable system with reduced stress, fewer disease problems and faster growth
- Cluster farming approach: For effective implementation of the biosecurity protocols, cluster farming approach is to be adopted.

At CIBA, consistently higher average production is achieved (2.5-3 mt/ha from a stocking of 12 /sq m) in the BZEST system compared to that of the control. In a particular experiment, 9 % gain in terms of production and 11 % reduction in FCR was achieved with shrimps registering better harvestable size (ABW-33 g) compared to that of the control (ABW: 30g). Similarly, semi-intensive farms in West Bengal managed through zero water exchange, stocked with healthy hatchery seeds, commercial feed and probiotic based farming yields 5-6 mt/ha shrimps.

Conclusion: This probiotic based farming system in all account could be very promising farming practices and in turn for the coastal ecosystem for its high scoring biosecurity measures and avoidance of antibiotics and objectionable chemicals. However, these techniques have to be standardized under Indian condition and only defined probionts or bioremediators in terms of its efficacy should be allowed to have environmental friendly and sustainable shrimp farming. Zero water exchange system is not stressful if managed properly with environment friendly chemicals and practices. Following a BZEST improved extensive farming system in tune with the directives of Aquaculture authority of India is recommended. Good water quality and lower stocking densities appear to be the best defense against all diseases. The greatest advantage of BZEST is effective biosecurity for the cultured animals and in turn for the coastal ecosystem.

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ORGANIC AQUACULTURE: STATUS, PRINCIPLES AND TECHNOLOGY

Akshaya Panigrahi and Shyne Anand

Introduction: Organic farming is often understood as a form of agriculture with use of only organic inputs for the supply of nutrients and management of pests and diseases. Indian experimentation with Green revolution slow down and the priorities in agriculture research are gradually moving from focus on individual crop performance to a total system productivity with due attention on product quality and environment safety (Rai, 2003). Aquaculture is the fastest growing food production system and doing it organic way will ensure sustainability and environmental, social, economical and institutional compatibility. So the solution to the declining productivity, environmental concerns and sustainability can be addressed by adapting to organic way of farming. Organic farming restricts the use of artificial chemical fertilizers and pesticides, chemotherapeutic medicines including antibiotics and encourages utilization of natural nutrients, probiotics and bioremedial measures. The merits of organic aqua-farming and how best the present traditional farms of West Bengal and other coastal states can be converted in to organic, the initiative have to be taken in that direction. The organic way of farming has to be popularized in this region and elsewhere so that it can reverse the depleting productivity, biodiversity, mangrove and other habitat in this region. CIBA have taken a lead in this regards and attempts to elaborate with all the vital questions to be attended before going organic. In this chapter we have discussed the definition, principle, present status of organic farming and more specifically the organic shrimp farming with the certification process and other prerequisites for moving forward in this direction.

Definition: Organic agriculture system is a farming system that maintains and enhances the health of soils, plants, animals and humans (IFOAM 2006). Organic aquaculture can be defined as a process of production of aquatic plants and animals with the use of only organic inputs in terms of seed and for the supply of nutrients and management of disease. However, the variety of species produced in aquaculture systems and vast differences in cultural requirements for finfish, shellfish, mollusks, and aquatic plants add to the complexity of defining this sector. Traditional farming, Sustainability, eco-friendly, and holistic, integrated approaches to production are hallmarks of organic systems.

Principles: Principles of organic agriculture were adapted to aquaculture and utilized. Organic aquaculture is being distinct from conventional semi intensive shrimp farming with

regard to complete prohibition in the use of artificial chemical fertilizer, pesticides, chemotherapeutics, and medicines including zero tolerance to antibiotics. A multi-productive system is created utilizing all the niche of the available resources i.e. the upper parts of the dykes planted in leguminous trees. Low food chain species easier for organic aquaculture as organic certification of these species that do not require fishmeal and oil is easier. Organic larvae, organic feed, stocking density limits, certified farming practices and post harvest practices all through eco-friendly operations are required for any organic aquaculture unit.

Organic certification: Organic certification is a process claim, not a product claim. In other words, organic standards regulate the practices and materials used to produce an agricultural product. Aquaculture includes all species and all stages and in all systems like extensive, intensive, semi-intensive or super-intensive, Out-door or In-door system, can be certified as organic. Based on this organic aquaculture can be classified as Certified organic aquaculture and Non-certified organic aquaculture.

Certified organic aquaculture: International organic aquaculture standards have been developed, many still in draft form, throughout the world. These include the one by Germany's Naturland, the UK's Soil Association, and Sweden's KRAV standards. The International Federation of Organic Agriculture Movements (IFOAM), a large umbrella organization, comprised of 750 organisations in 104 countries involved in organic agriculture from around the world has also drafted organic aquaculture standards and provides a platform and network for global exchange and co-operation on issues related to organic production. In India a charitable trust, INDOCERT, Kerala is the only nationally operating trust, accredited by Government of India for certification of organic farmers, processors and traders.

Non-certified organic aquaculture: Aquaculture that meets organic production standards, but is not subject to organic inspection, certification and labeling is referred to as "non-certified organic" as distinguished from "certified organic" Everywhere every system should have an opportunity to work under organic standards. Not all organic farmers are certified as such, even though they follow the principles of organic agriculture. In India organic aquaculture for the time being should not be limited to certified organic farms and products but includes all systems that involve natural processes, rather than all external inputs to enhance productivity. In that way the traditional farms could be converted to organic farm with little modification.

Present status: Presently, organic aquaculture production takes place primarily in developed countries especially in Europe, where certified organic salmon, carp, and trout are grown and

sold. Certified organic mussels, tiger shrimp, white shrimp, and tilapia also are cultured in countries like Vietnam, Peru, Ecuador, Chile, New Zealand, and Israel. Standards and certification procedures are set by just a few certification agencies discussed later. In India organic aquaculture is in very nascent stage. But one good thing is that from ancient time our farmers follow natural way to have the low input culture practice which is otherwise called traditional system of farming and this is close to the organic concept. Traditional organic farming systems rely on ecologically based practices, such as cultural and biological pest management, and virtually exclude the use of synthetic chemicals in crop production and prohibit the use of antibiotics and hormones in livestock production. Based on current estimates of certified organic aquaculture production and an anticipated compound annual growth rate of 30 percent from 2001 to 2010, it can be expected that certified organic aquaculture will increase considerably, while still remaining a tiny share of total aquaculture production. However, the quantities and diversity of certified organic produce being produced remain small (5000 mt of which 80 percent of salmon), partly due to the absence of universally accepted standards and accreditation criteria organic aquaculture. Worldwide very few shrimp farms are certified as organic and the first one is located in Ecuador which claims to translate "an aquatic desert to a biodiverse wildlife reserve...that also produces shrimp". After several years of initiation the farm was certified by Naturland (German) certification organization. Ocean Boy Farms in Hendry County will harvest in excess of two million pounds of organic Pacific White shrimp in second half of 2006. The company claim to produce highest organic shrimp in the world. Methods of production eliminate the need for hormones, antibiotics or chemicals to ensure the health of the growing shrimp and the organic certification and "bio-secure environment." is guaranteed. Again, Effective Microorganisms (E.M.) technology integrated into farming methods (organic), reviving nature with the power of living microorganisms.

Organic approach and mangrove conservation: The protection of the mangrove area (adjacent to the shrimp farm) is possible by adapting organic farming integrating the mangroves and making it certain it was saved by the same industry that once threatened it. It is possible that some of the abandoned shrimp farms will revert to mangrove forest in other parts of the country. Since a shrimp farm's ecological footprint will depend on the intensity of farming, it has been estimated to be as high as 35-190 times the size of the farm surface for a semi-intensive system, a low stocking organic system will have very less impact on the ecosystem. The ponds can be planted with mangrove trees found locally just at the water line of the ponds. The plants include *Rhizophora mucronata.*, *Avicennia alba.* The selection was

based on the salinity tolerance and other adaptation of the mangrove plants. The dykes of the organic shrimp pond can have vertical production zones. The lower part has different mangrove species, grasses, and aquatic plants, and the upper part has the leguminous trees, aloe vera, and fruit and flower trees. This may provide natural feeding areas for the shrimp.

Organic farming and Food security: By large scale conversion of the Pokkali in Kerala or Bheries in exports would vary depending on crop, but the structure of farming would definitely change with more diversification of aquaculture and inclusion of livestock and other species for optimise the nutrient utilisation and gap between the species.

Commercialisation and economic performance: Studies have shown that the common organic agricultural combination of lower input costs and favourable price premiums can offset reduced yields and make organic farms equally or often more profitable than conventional farms. Our study indicates that there is a reduction in cost of production and a higher rate of return and profitability in organic farming. Major profitability comes from the reduced FCR compared to the conventional farms and relative lower cost of organic inputs. As the organic inputs are used optimally the feed requirements reduced and also the use of yeast based organic preparations elicits the immune status of shrimp and has a role in enhancing the growth rate and protective response.

Social performance: The conversion of a farm to organic practices influences all facets of the operation, including labor demand, social structures, and decision-making processes. Gender equality and other social parameters are well taken care of incorporation of fair trade principles including fair wages, safe and healthy working conditions, and social services.

Institutional performance: Because "organic" is a production process claim, consumers must rely on certification programmes that verify claims. The standards that specify the organic production process appear quite precise, compared with the other production processes.

Technology for organic shrimp farming: CIBA's standpoint: The objective of organic aqua farming is to apply organic production practices to the entire life cycle of the animals with no breaks in the organic management. Under the framework given by IFOAM, CIBA is taking up the organic farming defining the principles and practices broadly under the following heads.

1. Aquatic Production systems:
2. Breeds and Breeding of Aquatic Animals
3. Aquatic animal nutrition
4. Aquatic animal health and welfare

5. Harvesting and post harvest care:

The Bheries in West Bengal. Gheries in Orissa and the Pokkali fields in Kerala are open system and still contribute highly towards the shrimp production in Asia. So the adoption of organic principles to the extent possible should be system specific if we want to cover more area of operation. CIBA have initiated the process with some parameters like low stocking density, organic inputs like manures and yeast based organic preparations. Zero tolerance to artificial chemical fertilizer, pesticides, chemotherapeutics, and medicines including antibiotics. Integration of mangroves and other plants in the organic ponds has been initialized. Organic density controlled shrimp farming is intended at least for the traditional farms whose production and yield quality could be improved under the organic banner. Judicious application of organic fertilizers including probiotic and yeast based organic preparations and vermicompost attributed a key role in optimizing pond productivity without adversely affecting the pond environment. The organic ponds maintained a higher gross primary production throughout the culture compared to that of the control ponds. CIBA have developed a low fish meal shrimp feed by incorporating plant protein, soyabean meal and demonstrated successfully in shrimp culture ponds with organic principles. Better quality & yield of shrimps with an average production level ranging from 1-1.5 mt with excellent FCR (< 1) is achievable from low stocking density (6/sq m), in this system. We have demonstrated that conversion of organic system leads to better or at least identical yields in extensive farming. In one particular experiment, the substantial gain in production level (17%) by following organic principles and 16% improvement in size at harvest (33.28g in organic compared to 28.64 g in conventional) with better FCR (lowered by 4.2 % in organic ponds). To be more specific in organic shrimp farming, the following aspects are emphasized.

- Certified hatchery
- All organic inputs
- Organic feed development
- Health management-organic way
- Commercialization of the produce
- Certification of farming procedures and processing

It is well conceived that setting up a organic shrimp farm is not possible in one go and we have taken a stand to go step by step before claiming the process and product complete organic. Research priority for developing or verifying technologies for the nutrient dynamics, use of probiotics, bioaugmentation and bioremediation process for shrimp aquaculture and

developing rapid and sensitive methods to detect pathogens within the animal and in its environment which will help in organic way of farming. In our case shrimp densities are low and this ensures not only good quality shrimps but even better effluent quality. The principles followed are universal principles which can be applied in any kind of farming system or land use pattern.

Conclusion: Organic aquaculture uses traditional and indigenous farming knowledge, while introducing selected modern technologies to manage and enhance diversity, to incorporate biological principles and resources into farming systems, and to ecologically intensify aquacultural production. It gives scope to the farmers to be innovative. Though aquaculture is expanding, the quantities and diversity of certified organic produce being produced remain small, partly due to the absence of universally accepted standards and accreditation criteria organic aquaculture and partly due to ignorance Organic aquaculture in general and organic shrimp farming in particular is bound to increase its stake. Greater government investment in appropriate research and extension services can help overcome constraints. India have to formulate specific standards and guidelines for organic fish products and eco labeling of wild catch as organic may also be encouraged after testing for antibiotic and pesticide residues. Organic farming is a progressive world view, a culture, and should be one main option for the future agricultural developments

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ADVANCES IN MOLECULAR DIAGNOSTICS AND THERAPEUTICS IN AQUACULTURE

R. Ananda Raja and Sujeet Kumar

Introduction

The use of nucleic acid-based techniques to detect the causative agents and to stop their replication in the host cells has gained importance due to their specificity and sensitivity. The techniques to identify the viral and bacterial pathogens affecting fish and shrimp published so far seems to be an ever-increasing rate for many years. Indeed, the adoption of these techniques has been much slower than had been expected. This chapter is aimed at not to provide an exhaustive review of all the methods available since it would quickly be dated and perhaps obsolete by the ongoing developments. So, it is focused to highlight the areas where molecular approach have been successful. some lacunae that they have not yet been adopted as expected and some future developments in the molecular therapeutics.

Polymerase Chain Reaction (PCR)

The structure of the DNA is described by Watson and Crick in 1953, which led to the further development in the molecular biology. It has been the most significant achievement for the molecular diagnosis after the advent of PCR. The *in vitro* amplification of DNA and generation of complementary DNA (cDNA) by reverse transcription of RNA is done several years prior to the invention of PCR. Mostly, short stretches of nucleic acid that are unique to the target organisms are amplified and its presence considered as a sufficient evidence for the presence of target pathogen. There is a possibility of PCR primers cross-react with other closely related organisms, giving false positive results. The chances of false positives can be reduced through judicious primer selection. The use of nested PCR protocols can further improve the sensitivity of detection. With a novel or poorly studied pathogen, post-amplification analyses improve the specificity of the test.

Post-PCR Analysis

The product can be further analyzed with other amplification methods like nucleic acid sequence-based amplification (NASBA) which has advantage over conventional PCR but has not yet been widely applied in diagnosis of aquaculture related diseases. Random amplified polymorphic DNA (RAPD) is a modification of PCR that can potentially scan the whole genome to reveal variation, rather than targeting a small portion for examination and is used in developing diagnostic tests for fish pathogens. The pairing of nucleotide bases facilitates the use of fragments of DNA that will hybridize to its complementary sequence.

Labeling the probes allows their detection where colorimetric, fluorescent and chemiluminescent visualization have replaced the radioactive methods. In Southern blot, the genomic DNA is digested with a restriction enzyme and separated by gel electrophoresis. Then the specially designed probes are used to identify the DNA. Restriction fragment length polymorphism (RFLP) reveals the differences in sequences due to gain or loss of recognition sites for restriction endonuclease enzymes. Other methods such as Single-stranded conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and RNase protection assay (RPA) can also demonstrate single nucleotide variations between fragments of nucleic acid of the same length. All these methods and application of probes can confirm the specificity of the PCR product. Sequencing provides the greatest level of detail in analysis of genetic material from a pathogen by exhibiting order of four bases in a fragment of DNA. Its application in developing molecular methods for diagnosis and in epidemiology is highly commendable. Future innovation in hardware may bring sequencing in to the range of rapid and easy diagnostics like sequencing based on real-time pyrophosphate.

New Molecular Techniques

The ultimate aim of new tests is usually to improve the sensitivity and/ or specificity of diagnosis. The analysis of nucleotide sequences can provide much more detailed information on a pathogen than its phenotypic study. So, new molecular techniques have been developed for all economically important pathogens of aquaculture interest. But, their wide application to the level of expectation is limited based on the satisfactory performance at field. Typing isolates by analysis of the small subunit, or 16S, ribosomal RNA genes appears a useful tool as *Streptococcus iniae* is identified from human patients. The addition of data from molecular diagnostics can assist epidemiological analysis of disease outbreaks and disease management. The techniques such as *in situ* hybridization allow assessment of the location of the pathogen within the tissues of the host. The ability to detect fish carrying sub clinical levels of pathogens is a great advantage with the molecular diagnostics Carrier fishes need not be reared to maturity and they could be removed from a population and reduce the risk of pathogen spread. The ability to test fish through non-lethal sampling of mucus, blood or biopsy is a significant benefit offered by molecular methodology. Nucleic acid amplification can be combined with antibody binding to amplify a signal and improve sensitivity of detection. Other amplification methods like rolling circle amplification and real-time PCR may become more popular in disease diagnostics in near future. Many advances in methods and equipments are developed and applied first in research before being adopted for routine diagnostic use. Microarrays are one among them. Microarrays are mainly used in

genes and protein expression studies and are also being applied in proteomics. The use of microarrays can assess many genes or polymorphisms at once, providing a more detailed picture of the organism. Microarrays will dramatically alter the speed and scale of molecular analysis of pathogens. Arrays are now being applied using antibody-antigen binding and these open up another avenue for analysis, alongside host-pathogen interactions. The other major advance in the near future is likely to be the development of "laboratory-on-a-chip" devices that will enable on-site molecular detection and analysis. Further electronic chips could carry out amplification and hybridization and if these devices can be fabricated in a robust format, they could permit analysis on-site that currently requires several days and man-power in specialized laboratories.

Molecular techniques and their greater sensitivity

The ability to discriminate different strains of virus or bacteria provides new opportunities for controlling only harmful types. Equipments, reagents and practices vary between laboratories and may require modification before a test performs satisfactorily. There have been many instances of a single protocol performing differently in different laboratories. The fact that a single protocol may not suit all laboratories should not prohibit the application of different methods in different situations or locations. Indeed, application of more than one test may even instill greater confidence if the results concur. So, there is no substitute for practical experience with a method for instilling confidence in the technique and in the interpretation of its results. It should be remembered that not all PCR primer sets or methods will perform equally well. Henceforth, it warrants comparison of different methods to provide some validation. Conducting inter-laboratory or ring testing will be an excellent way of examining inter-laboratory or inter-test performance. During this process, great care must be taken to ensure that test materials sent to different laboratories are suitable and comparable; this is often the greatest challenge in setting up inter-laboratory comparisons. This will pave way for accreditation and quality assurance for the particular lab and its protocol.

On farm use of Molecular Techniques

The application of molecular diagnostics is recently gaining acceptance and popularity. A rapid diagnosis should be more vital for appropriate response and treatment for a fish and/or shrimp population than terrestrial animals. If culture of an organism is required, the time lapsed between the sampling and the results can allow spread of the pathogen to entire population. So, a swift identification is needed to preventing infection and total loss of production. Molecular testing provides significant advantages with less time. Future

developments in technology and methods are likely to provide probes, dipsticks, or hand-held thermocyclers that can be employed for an on-site diagnosis.

Bottlenecks in the Application of Molecular Diagnostics

There are some limiting factors that presently restrict large-scale applications or throughput. Scaling up PCR, hybridization and sequencing has been possible through the use of 96-well formats to be economical. Phenol/chloroform-based methods for nucleic acid extraction perform well but are laborious and involve harmful chemicals. Various columns are produced commercially for extraction of DNA and/or RNA from a variety of starting material. If suitable, these kits can greatly improve the ease and efficiency of extraction. Before they are adopted, care must be taken to ensure that they provide equivalent yields of nucleic acid to other methods and to prevent cross-contamination, which is always a prime concern in clinical diagnostic testing. Gel electrophoresis and other methods of analyzing nucleic acids can also restrict throughput. These problems have largely been overcome in systems such as real-time PCR which avoid the use of gels altogether. Initially, molecular tests were largely developed in laboratories that were devoted mainly to research. It needs closer cooperation of research and diagnostic groups to ease the transfer of technology at field level. The establishment of any diagnostic facility requires considerable investment in laboratories, equipment and trained personnel. The costs of maintaining such facilities are escalating as there is increased demand and pressure for these laboratories to be accredited and maintain Quality Assurance systems. Finally, as molecular diagnostics have gradually developed and increased in popularity, peer pressure will also influence decisions to adopt these techniques. It may be more economically viable to have centers of excellence for certain pathogens or techniques, and to refer samples to these centers instead of having expensive equipments duplicated in various laboratories without regular use. Concern over specificity of results is often a major obstacle for the adoption of PCR or probes for fish pathogens. It has been claimed that not enough is known about other organisms that may be present in the sample but are not pathogenic, yet may cross-react with primers or probes to yield a false positive result in a molecular test. So, thorough knowledge of the type of organism the test aims to detect, together with information on the variability of the genome regions being targeted, will guide an appropriate choice of primer or probe.

Molecular Therapeutics

An understanding and exploring the natural antiviral immune mechanism induced by dsRNA will bring a solid conceptual framework for the development of strategies to control viral diseases in shrimp aquaculture. Recently, it is proved in shrimp that *in vitro* synthesized

dsRNA induces a general antiviral response. It may be either sequence-specific or sequence-independent (innate immunity) manner as reported against three unrelated viruses such as White Spot Syndrome Virus (WSSV), Taura Syndrome Virus (TSV) and Yellow Head Virus (YHV). The molecular basis for sequence independent immunity and the presence of Interferons (INFs) as in vertebrates remains elusive with the available knowledge on genomics and proteomics in crustaceans. Further, it is proved that siRNAs are less capable of inducing innate immune response than dsRNA in shrimp, *Litopenaeus vannamei*. So, it can be concluded that the product size of RNA plays a role in determining the anti-viral immunity. The ability of the cells, *in vivo*, to detect and internalize extracellular dsRNA to initiate intracellular gene silencing phenomenon implies the existence of cell surface receptors that mediate the uptake of dsRNA. It is found that dsRNA travels probably in the circulation from the site of injection to distant tissue and evinces highly sequence-specific gene silencing. But, it is not economically feasible to synthesize *in vitro* dsRNA and siRNA in large quantities for RNAi therapy in shrimp culture ponds. As an alternative, production of bacterially expressed virus specific dsRNA will enhance the large-scale production of dsRNA for field application. The ultimate identification of gene responsible for RNAi will bring out exploration of this natural immunity in shrimp. But, question arises if RNAi is a natural antiviral immune mechanism, then at least some viruses should have evolved strategies to suppress or evade this phenomenon. Thus, it is proved that WSSV has more RNAi suppression than TSV as evidenced by loss of RNAi mediated down-regulation of STAT (Signal Transducer and Activator of Transcription) mRNA in WSSV infected shrimp. So, it is important to understand anti-RNAi functions of the virus like WSSV in shrimp.

Conclusion

The application of molecular diagnostics and therapeutics have incurred more intense scrutiny and calls for validation than any other methodologies. Perhaps, this is due to the difficulty of validating a test that is more sensitive than any other. The application of molecular methods in diagnostic testing offers ever-increasing advantages as further techniques and equipments are developed. Yet the adoption of molecular testing for fish and shellfish has, overall, been much slower than expected. Several factors will promote the use of molecular diagnostics and these should be encouraged wherever possible. Data from validation trials should be made available, if not via traditional publications then at least through release on websites, etc. This will prevent duplication and allow ready assessment of suitable methods to adopt for each particular circumstance. The use of parallel testing should not be underestimated and should be readily accepted as a means of validation for molecular

diagnostics. After the advent of successful antiviral molecular therapeutics, appropriate drug delivery methods for complex and dynamic aquaculture system further challenges and needs long run to go. It is expected that further airing of optimistic approach in adoption of molecular diagnostics and therapeutics will promote further the advancement of this field overcoming the present bottlenecks.

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VACCINE IN AQUACULTURE

Sujeet Kumar and R. Ananda Raja

Introduction

Vaccines are antigen preparation derived from a specific pathogen and stimulate the immune system in such a way to generate resistance to same pathogen from subsequent infection. Specificity and memory of the adaptive immune system are two key elements exploited in vaccination. An effective vaccine must be safe, immunogenic and protective.

Type of Vaccine

Success of any vaccination program depends upon types of vaccine available, its efficacy, safety, duration of immunity as well as its final cost. Currently, in aquaculture four types of vaccines are available.

1. Killed Vaccine
2. Live Vaccine (Attenuated vaccine)
3. Subunit Vaccine
4. DNA Vaccine

1. Killed Vaccine: This is the most commonly used vaccines in aquaculture. Heat or formalin is commonly employed for inactivation of bacteria, while, formaldehyde, glutaraldehyde, binary ethylenimine and β-propiolactone are used as inactivating agent for viral vaccines.

Advantages

a. Very effective in inducing humoral antibody response so effective against most of the bacterial pathogens.
b. The vaccine is safe as the microbes are in killed form and can't revert to virulence.
c. Can be stored at room temperature.

Disadvantage

a. Poor cell mediated immune response so not very effective against intracellular pathogens such as virus.
b. Use of adjuvants and sometimes booster vaccination.
c. Use of inactivating agents which may alter the form of a critical antigen, and therefore reduce vaccine effectiveness.

Successful commercial killed vaccines are available against Vibriosis (*Vibrio angullarum, V. Ordalli)*, Enteric red mouth disease for salmonids (*Yersinea ruckeri*), Furunculosis for salmon (*Aeromonas salmonicida*) and spring viremia of carp.

2. Live Vaccine: Live vaccines or attenuated vaccines are mutated strains of an infectious agent that have a reduced or no ability to cause disease. Such type of vaccine is highly desired for intracellular pathogens such as virus.

Advantages

a. Long lasting immunity even in small doses.
b. Strong cellular immunity thus very effective in protection against intracellular pathogen such as viruses.

Disadvantages

a. Capacity of vaccine strains to revert to a virulent form and potential to cause disease in immuno suppressed host. By methods of genetic engineering now it is possible to irreversibly attenuate the microbes by removing virulent genes.
b. Risk of transmission to non-farmed fish in the surrounding water for which the vaccine may be virulent.
c. Cold chain maintenance for storage.

Live vaccine is available against Bacterial kidney disease using related bacterium *Arthrobacter davidnelli*. Edwardsillosis (ESC) using RE-32 strain of *Edwardsiella ictaluri* and aroA and purA deficient *E. Ictaluri* strain.

3. Subunit Vaccine: Subunit vaccine includes immune response against the purified protein, synthetic peptides and recombinant protein (recombinant vaccine). For the subunit recombinant vaccines, the gene(s) encoding a particular antigen, which must be immunogenic, are cloned and subsequently introduced into a permissive host, e.g. bacterium, yeast or insect cells which then synthesize the recombinant antigen.

Advantages

a. Well-defined, non-infectious and inexpensive to produce in large quantities.
b. Differentiation of vaccinated from potential carrier by immunological methods is possible because fish is not exposed to the entire array of antigens that a pathogen expresses.
c. Useful for pathogens which are difficult to bulk culture, such as viruses.

Disadvantages

a. Need of adjuvant and vaccine delivery system
b. Larger dose and need for booster immunization
c. Shorter duration of immunity
d. Poor cell mediated immune responses

Licensed recombinant vaccine for fish is available against infectious pancreatic necrosis (IPN) using VP2 gene of the virus. Glycoprotein of infectious hematopoietic necrosis (IHN) and viral hemorrhagic septicemia (VHS) virus have shown good results in experimental trials.

4. DNA Vaccines: Genetic immunisiation using naked DNA is most recent approach in vaccine design. This technique involves injection of naked DNA directly into the skeletal muscle of the fish where it is expressed extra chromosomally. Gene gun, electroporation, encapsulation of DNA in liposomes or polylactide-L-glycolide (PLG) microparticles enhances the cell uptake of DNA vaccine. Incorporation of CpG motifs or cytokines to plasmid expressing DNA vaccine creates a cytokine microenvironment conducive for developing an adaptive immune response.

Advantages

a. Easy to manufacture: DNA vaccine is comprised of a plasmid with origin of replication, a selectable marker and the gene of interest under a strong promoter. This single platform makes DNA vaccines very attractive from the prospective of manufacturing.
b. Safety: DNA vaccines are considered safe since it lack extraneous materials and are non-infectious. The chance of integration into host genome is also less as proved by many experiments.
c. Multicomponent vaccines: Combining many plasmids encoding different genes of interest or introduction of two different genes in a single plasmid is also possible. This paves the way for vaccination against many diseases at single time in single stroke.
d. Less vaccine dose: Fish rhabdoviruses such as Infectious hematopoietic necrosis virus (IHNV), Viral hemorrhagic septicemia virus (VHSV) a single IM injection of a 1.0 µg with no adjuvant or boosters, was found sufficient to provide a high level of protection (Kurath, 2008).
e. Induction of both humoral and cellular immune response.
f. Potent inducer of immunity in neonates in absence as well as presence of maternal antibodies.

Disadvantages

a. Sub-optimal immunity: DNA vaccine has poor transfection efficiency leading to suboptimal induction of immunity.
b. Licensing and commercialization of DNA vaccines is difficult partly due to public perception who confuse it with genetically modified organisms, and stringent law.

c. May prove hazardous due to widespread release into aquatic environment which lack physical and physiological barrier.

Recently, a DNA vaccine was licensed to immunize fish against infectious hematopoietic necrosis virus for commercial use in Canada (Babiuk, 2008).

Vaccine Delivery System

Three different methods of administration are commonly used to vaccinate fish, namely injection, immersion and oral.

Injection Administration: Injection of antigens (intraperitoneal or intramuscular) is an effective way of provoking antibody response in fish by maximum retention of vaccine in the body. However, procedure is labor intensive and stressful to fish and suitable only for larger fishes like broodstock and not fit for fish below 15 grams.

Immersion Administration: Immersion of fish in antigen solutions has emerged as potent commercial process. Prior immersion of fish into hyperosmotic salt solution enhances the antigen uptake. The method is less labor intensive and less stressful to fish compared to injection method. It can easily be used to vaccinate small fish, while larger fish can also be vaccinated by spraying.

Oral Administration: Oral administration is "the ideal method" for administering vaccines to fish where the vaccine is incorporated into fish feed. It is least labour-intensive, avoid handling stress and can be used to vaccinate large numbers of fish of all sizes. The major limitation is lower levels of protection which is due to degradation of antigen by the gastric fluid and inefficient transport of antigen across the gut wall. Microencapsulation and bioencapsulation using live artemia has shown good results.

Fish Vaccination Research

Warm water aquaculture in Asia has problems with several bacterial diseases such as motile Aeromonad septicaemia or hemorrhagic septicemia, Vibriosis, Columnaris, Edwardsiellosis etc.

Hemorrhagic septicaemia is caused by motile species of Aeromonas such as *Aeromonas hydrophila, A. sobria, A. caviae etc.* Currently no commercial vaccine is available against hemorrhagic septicemia. Furunculosis in salmon and turbot is caused by non motile *Aeromonas salmonicida.* Oil adjuvant vaccine is commercially available against Furunculosis which provides lifelong protection.

Vibriosis is caused by *Vibrio anguillarium.* A widely acceptable formalin inactivated whole cell vaccine is available against Vibriosis which gives very good protection by immersion methods.

Columnaris disease is caused by *Flexibacter columnari* which affects both warm and cold water fishes. Recently a live vaccine based upon rifampicin resistant *Flavobacterium columnare* strain has been patented for commercial use.

Edwardsiellosis caused by *Edwardsiella tarda*, seriously affects carp, tilapia, mullet, catfish and eel culture. Till now no commercial vaccine is available for *E. tarda* due to variability in serotypes. Edwardsiellosis caused by *E. Ictaluri* affect channel cat fish culture. Recently two live vaccines for *E. Ictaluri* based upon RE-32 strain and aroA and purA deficient strain has been licensed for commercial use in channel cat fish.

The impact of viral diseases on fish in India is largely unknown. In cold water fish culture, several viral diseases such as infectious hematopoietic necrosis, viral hemorrhagic septicaemia, infectious pancreatic necrosis etc. have been recorded. A DNA vaccine using glycoprotein gene against infectious hematopoietic necrosis (IHN) virus and recombinant vaccine using VP2 protein against infectious pancreatic necrosis virus has been recently patented.

White spot syndrome virus (WSSV) and other viral diseases became serious threat for shrimp industry worldwide. It is generally thought that shrimp lack the immunoglobulin based adaptive immune system. However many experiment exhibited the presence of 'quasi-immune response' in shrimp. Recombinant protein and DNA vaccines taking viral structural protein, VP28 has shown promising results on experimental trial.

In Indian context challenges lies to develop effective vaccine for cultivable fresh water fishes against *Aeromonas hydrophilla* and for cultivable marine shrimp against *Vibrio* and white spot disease virus.

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BIOREMEDIATION MEASURES AND PROBIOTICS IN AQUACULTURE

Sujeet Kumar, Akshaya Panigrahi and R. Ananda Raja

Introduction

In any sphere of health management, prevention is indeed better than cure. In nature, there exist a delicate balance between the host, pathogen and environment. Disease outbreaks occur when this delicate balance gets disturbed. Recent techniques of intensive and super intensive culture practices in aquaculture can stress the host, favors the pathogen, and result in disease outbreak. Various preventive measures such as probiotics, immunostimulants, and other bioremediation products are now in constant use in hatchery and farm operation to maintain the better animal's environment and to prevent the occurrences of disease.

Use of bioremediation in aquaculture

Bioremediation is a process of reducing hazardous wastes to environmentally safe levels through the use of microbes.

a) Bioremediation of detritus: Due to continuous accumulation of organic matter at the pond bottom, anaerobic condition generated. In this situation soil microbes revert to anaerobic respiration and produces obnoxious gases such as H_2S, NH_3^+, N_2, H_2, and CH_4. Bacteria like *Bacillus subtilis and B licheniformis* are used for faster degradation of detritus (organic matter). These bacteria are capable to work efficiently in anaerobic condition as it can use NO_3 as an electron acceptor in absence of oxygen.

b) Bioremediation of Ammonia: The most obvious use of bioremediation in aquaculture is the use of biofilter which favours nitrification by the immobilized *Nitrosomonas* and *Nitrobacter* on substrate. These nitrifying bacteria uses NH_4^+, NO_3 and NO_2 as an energy source. Aquamats developmed in shrimp culture for water purification is based on this principle. Bacterial film promoted on biodegradable substances such as sugarcane bagasse has been observed to markedly reduce toxic ammonia level in shrimp as well as carp culture system.

c) Bioremediation of Hydrogen sulfide: hydrogen sulfide is toxic to aquatic animals as it binds with enzymes and blocks oxidative process. Anoxyphotobacteria (Purple sulphur bacteria, Green sulphur bacteria and non sulphur photobacteria) splits H_2S into elementary sulphur and hydrogen ion. They grow at sediment water interface and are efficient mineraliser as they grow in both anaerobic and aerobic conditions even in dark without using solar energy.

Probiotics

Probiotics is defined as a live microbial feed supplement, which is often introduced into the food chain to shift the microbial balance from disease causing microorganisms to beneficial microorganisms.

Types of probiotics

Probiotics are mainly of three types

a) Gut probiotics or feed probiotics: It can be blended with feed and administrated orally to enhance the useful microbial flora of the gut.

b) Water probiotics: This proliferates in water medium and excludes the pathogenic bacteria by competitive exclusion for nutrients.

c) Soil probiotics: Soil probiotics are generally used as bioremediation product.

Methods of application of probiotics

Probiotics are marketed in two forms.

a) Dry forms: Dry probiotics come in packets and can be used with feed or applied to water. It should be incubated or brewed at 27–32°C for 16 to 18 hours with continuous aeration before application.

b) Liquid forms: The hatcheries generally use liquid forms which are live and ready to act. These liquid forms are directly added to hatchery tanks or blended with farm feed.

Desired quality of a probiotics

A good quality probiotics should have following characteristics.

- Must not be harmful to the host.
- Should be effective over a range of temperature and variations in salinity.
- Able to grow and survive in the intestine.
- Useful to the hosts in growth promotion, food utilization and improvement of health.
- Capable of being propagated as a viable product in large scale.
- Remain stable and viable under different storage conditions.

Assessment of potential probiotic candidate

Methods to select probiotic bacteria for use in the aquaculture should include the following steps:

- Collection of background information: Background information regarding culture practices and economics should be collected to evaluate whether a probiotic application would be feasible or not.
- Acquisition of putative probiotics: A large pool of putative probiotics should be isolated from the host or culture environment.

- Screening of putative probiotics: Putative probiotics are screened by *in vitro* antagonism tests. Candidate probiotics can also be selected based on production of inhibitory compounds like bacteriocines, siderophores etc.
- Evaluation of pathogenicity and survival test: Probiotics should not be pathogenic to the hosts and this should be confirmed prior to acceptance. The probiotics strain should have efficient adherence to intestinal epithelial cells to reduce or prevent colonization of pathogens.
- In vivo evaluation: Effect of candidate probiotics should be tested *in vivo* which involves introduction of probiotics into the host and evaluation of growth, colonization, survival and physico-chemical parameters.
- Effects in rearing conditions: Pond experiment should be conducted to conclude that the strains are beneficial.

Mode of action of probiotics

Different probiotic bacteria act differently. Following mode of action has been observed:

a) Production of inhibitory compounds: Probiotic bacteria release a variety of chemical compounds that are inhibitory to both gram-positive and gram-negative bacteria. These include bacteriocins, siderophores, lysozymes, proteases, hydrogen peroxides etc.

b) Competition for adhesion sites: Probiotic organisms compete with the pathogens for the adhesion sites and food in the gut epithelial surface and finally prevent their colonization

c) Competition for nutrients: Probiotics utilizes nutrients otherwise consumed by pathogenic microbes.

d) Source of nutrients and enzymatic contribution to digestion: Probiotic microorganisms supply food component such as fatty acids and vitamins to the host. In addition, some bacteria may produce extracellular enzymes, such as proteases, lipases, as well as necessary growth factors

e) Enhancement of immune response: Probiotics may work by stimulating non specific immune mechanism like increasing phagocytosis and antibacterial activity. *Bacillus* sp. (strain S11) has provided disease protection by activating both cellular and humoral immune defenses in tiger shrimp (*Penaeus monodon*).

f) Influence on water quality: Probiotics bacteria help to improve the water quality in aquaculture ponds. This is due to the ability of the probiotic bacteria to participate in the turnover of organic nutrients in the ponds. Nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter* are used to remove toxic NH_3 and NO_2

g) Interaction with phytoplankton: Probiotic bacteria have a significant algicidal effect on many species of microalgae. Bacteria antagonistic towards algae would be undesirable in green water larval rearing technique in hatchery where unicellular algae are cultured, but would be advantageous when undesired algae species are developed in the culture pond.

h) Antiviral activity: Some bacteria used as candidate probiotics have antiviral activities.

Current status of probiotics use in aquaculture

Probiotics used widely in shrimp and fish culture. *Streptococcus* and *Lactobacillus* have been used for rearing of live feed (rotifer) and *Flexibacter* for rearing of Artemia. *Vibrio alginolyticus* from a shrimp hatchery used in salmon culture effectively reduced disease caused by *Aeromonas salmonicida*. Probiotics are used in cultivation of shrimp larvae. Some of the probiotics like non pathogenic isolates of *Vibrio alginolyticus*, *B. subtilis* etc. are inoculated into shrimp culture with an aim to suppress the pathogenic vibrios, such as *Vibrio harveyi*, *V. parahaemolyticus* and *V. splendidus* thereby reducing the problem of opportunistic invasion by these bacteria.

Future perspectives

Though several studies have shown that the probiotic has potential in the aquaculture sector, much work is still needed in the following direction:

- Study on effectiveness.
- Mechanism of action.
- Potential of reverting pathogenicty to the host.
- Diffrentiation of probiotic bacteria from pathogenic bacteria.
- Side effects to environment.

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BIOSECURITY MEASURES AT FOREFRONT: TIPS TO PREVENT WHITE SPOT DISEASE IN SHRIMP AQUACULTURE

Akshaya Panigrahi, R. Anand Raja and Sujeet Kumar

Measures to combat diseases of tiger shrimp (namely MBV-Monodon Baculo Virus, YHD yellow head disease, WSSV-White spot syndrome virus disease, TSV-taura syndrome virus among the viral disease and vibriosis among the bacterial disease) are assuming high priority as it has caused significant economic losses in Asia-specific. At present twenty viruses has been identified as important to shrimp, the most threatening one being WSSV in Asia and TSV in America. Again multiple viral infections by HPV, IHHNV and MBV were also observed. Again, one (IHHNV infection) has been reported to interfere with other (WSSV infection), thus making it complex to understand. Recently disease status of exporting and importing country in terms of past health history documentation and on-going monitoring program assume importance as per agreement of the world trade organizations. All these concerns brought biosecurity (defined as the sum of all procedures in place to protect living organisms from contracting, carrying, and spreading diseases and other non-desirable health conditions) to the forefront of hatchery and farm management.

Again the importing country have placed import ban on shrimps containing antibiotic residues and discarding the total consignment. Antibiotics not only help develop antibiotic resistant strain of pathogens but also cause immunosuppression in shrimps. Similarly chemotherapeutics followed do not have any scientific basis. We must go for alternative health management approach for eco-friendly and sustainable shrimp farming. New approach in health management: system management approach (SMA) to aquatic animal health is to be practiced which involves a broader ecosystem management against introduction of pathogen.

Recent developments in shrimp health management includes-

1. Biosecurity and HACCP compliance
2. More efficient and cost effective use of inputs- water, seed and feed
3. SPF and high health lines
4. Good nutrition.
5. Harnessing host's nonspecific defense mechanism to reduce susceptibility to disease.
6. Use of probotics and bio-augmentation for the improvement of host and environment.
7. Viral accommodation and other passive immunization strategy explored towards vaccination !!!

8. Effluent treatment and discharge through bio-pond

The white spot syndrome is a disease caused by a family of related viruses named as white spot syndrome virus. The WSSV has been established as the "necessary cause" of WSD, though presence of the necessary cause alone will not lead to a WSD outbreak in a pond. All decapod (Order Decapoda) crustaceans (shrimps, prawns, crayfish, lobsters and crabs) from marine, brackish, or freshwater sources are potential hosts for WSD. This was first reported in Taiwan and mainland China in 1991-92, and subsequently found in many Asian countries including Japan, Indonesia, Republic of Korea, Malaysia, Thailand, Vietnam, the Philippines and India.

Symptoms include shell spotting from abnormal deposits of calcium salts, and occasionally a reddish discoloration due to expansion of cuticular chromatophores. When farmed shrimp are infected, they become lethargic, stop feeding, swim slowly near the pond surface, and eventually sink to the bottom and die. Shrimp mortality can reach 100%.

Fig: White spots visible on cephalothoracic shell of WSD infected shrimp compared to the normal animal

There is no successful therapy for viral infection; prevention is the only viable strategy.

Tips for White spot virus prevention and treatment-

In hatchery system:

- Seawater must be filtered (sand filter/net filter), precipitated overnight, disinfected with 30 ppm calcium hypochlorite (containing 65% active chlorine) for 12 hours neutralised by soda ($Na_2S_2O_3$, at 30 gm/m3) and aerated prior to use. Filtered water can also be treated with ultraviolet radiation or ozonated (UV irradiation must reach >30 000 mws/cm2 in the incoming water flow, while the ozone content in water must be more than 0.5 μg/ml for 10 min for effective disinfection from viruses (including WSSV), bacteria, fungi and protozoa.
- Prevent infection during transportation. Broodstock from different sources should be separately kept and care taken to avoid cross contamination. Quarantine procedure should be strictly followed
- Select virus-free broodstock as vertical transmission of WSD can occur from broodstock to offspiring through infected oocytes. Broodstock should be screened with sensitive diagnostic technique such as nested polymerase chain reaction (PCR).
- Do not transfer or import broodstock or larvae from other country without health certification

- Good sanitary measures including personnel hygiene, foot-bath filled with 200 ppm iodophore, lysoi or any other suitable disinfectant and for hands bottles containing povidone iodine (20 ppm and/or 70 percent alcohol) to be used at the entrance.
- All hatchery tools including nets and glasswares should be dipped in chlorine (500 ppm), muriatic acid (10 percent), potassium permanganate (KMnO4,20 ppm), formalin (200 ppm) or hydrogen peroxide(20 ppm) for 5 minutes before and after use. Pipes with biofilms should be subjected to remedial measures. Airline pipes should be fumigated with formalin and/or alcohol in the same way.
- Avoid feeding broodstock with trash fish (raw or frozen) including crab, other crustaceans and clams which may be potential carrier of white spot virus. It must be steamed before being fed.
- Wash the eggs properly, the surface of which may be contaminated. It can significantly reduce the chances of infection. Dipping the collected egg in 5 ppm calcium hypochlorite for 5 min or 50 ppm povidone iodine solution for 1 min followed by cleaning in sea water for 5 mins.
- Maintain optimum stocking density during larval rearing starting with 150 000 - 200 000 nauplii/m3 and good water quality and remove organic waste including the left out feed and larvae
- Quality food in appropriate dose and frequency
- Avoid any stressors like sudden temperature, salinity fluctuation

In grow-out system

- Evolving culture practices addressing biosecurity threats like that of closed systems and zero water exchange systems are preferred as WSD is widespread along the coastal waters. Every drop of intake water must be disinfected with 30 ppm calcium hypochlorite and left for 3-4 days. Minimize the water exchange in case of open system.
- Prepare the pond properly-the sludge after harvest contains high load of organic matter, toxic compounds, bacteria, parasites, virus particles as well as many WSD virus carriers. Effective pond preparation include black material removal by washing with high pressure water or scraping, drying for at least two weeks to kill all disease causing organism such as fungi, protozoa, bacteria and viruses by oxidation
- Eradicate virus carriers as wild shrimp, crabs, mysids, copepods and other crustaceans can one; screen water with nylon screen of 60-80 meshes/cm^2, placed as three tier

filtration at the inlet of the reservoir. Many animals like mudskippers, snakes, frogs could be out of farm by installing a fine net enclosure.

- The WSSV can enter the shrimp and pond through different routes, including shrimp seed, water, carrier animals and transfer of infected animals and farm equipment from one farm to another.
- Stock virus free post larvae- check it at reliable laboratory; go for quality check by stress tests, no wild seeds. Optimize stocking density and avoid very high density. Acclimatize the PL properly before release in pond at dawn or dusk.
- Avoid excess feeding and old feeds; use check tray and monitor feeding as per the standing biomass and physiological condition of shrimp. Restricted feeding during molting. Never feed live crustaceans or trash fish and its frozen product. Steam or boil before it is fed to shrimp, but pellet feed with balanced nutrients are desired.
- Oral immunogens like probiotics and a number of effective immunostimulants help improve the host immunity by eliciting the non specific immune response as shrimps lack specific immune response
- Avoid stressful conditions-low water depth, overcrowding, high temperature conditions and poor water quality, any other bacterial or protozoan build up- or factors that positively influence growth of secondary pathogens. Bacteria and fungi are opportunistic and can be easily dealt with by management or therapy.
- Water quality monitoring and management with application of lime, gypsum. Temperature, no aeration, algal bloom or crash, toxins and disturbing pH may increase the susceptibility of WSD. Low salinity may reduce WSD infection.
- Application of probiotics/immunastimulants could be considered if the effect is visible and economics allows. Immunostimulants incorporated into feed may enhance resistance of shrimp. Peptidoglycan and beta glucan are also found to give resistant against white spot diseases.
- In addition, nutritional supplements such as vitamin C and some herbal preparation *Phyllanltus* spp, *Calotripus gigantica* are reported to help
- Regular health monitoring and PCR testing of white spot virus and other infection should be done
- Virus can be inactivated by halogenous disinfectants including sodium hypochlorite or formalin - 0.25%, 0.5 ppm chlorine and 0.3 ppm Iodine
- Treat pond effluents as per the norms set by aquaculture authority of India treatment of effluent is mandatory for bigger farms and collectively for smaller farms. This

includes disinfection or biological filtration through cultivation of algae, sea weeds, clams, and filter feeders or omnivorous fishes to reduce the excess organic matter and pathogenic microorganisms.

Selecting disease resistant species is one important criterion though the SPF stock for cultivable species is limited. If WSD is persistent with *Penaeus monodon* in a certain locality, it is recommend that banana shrimp *P. merguiensis* which is relatively more resistant to WSD or finfish like *Lates calcarifer* be cultured for a few cycles. In spite of taking all these measures if still WSD appears, steps to minimize loses should be undertaken. In case the symptoms of WSD appeared within a month, shrimps are anorexic and dying kill the stock by disinfecting with Calcium hypochlorite and discharge after 7 days. But WSD appears in 2nd month or beyond care should be taken to minimize the damage. Iodine (0.3 ppm-repeated application) or chlorine at 0.5 ppm, help in arresting or inactivating the viral growth. As cannibalism of the dead shrimp causes multiplication of virus in pond they should be promptly removed. However there is no successful therapy for WSD as of now.

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BETTER MANAGEMENT PRACTICES: CONCEPT, PRINCIPLE AND COASTAL AQUACULTURAL SUSTAINABILITY

Akshaya Pnigrahi and Shyne Anand

Aquaculture has evolved as the fastest growing food producing sector in the world and an important component in food security and shrimp farming has significant contribution to it. However, worldwide shrimp farming activity is regressed by diseases and unsustainable practices which sometimes affecting the coastal ecosystem and livelihood. There is a paradigm shift from intensive system of shrimp farming to low input, environment friendly, sustainable culture practice through best management practices and the setting up of aquaculture authority to look after the environmental concerns, makes it imperative to give more importance for ecobased farming systems. System specific and cost-effective, better management practices (BMPs) is being developed for sustainable coastal aquaculture focusing more on shrimp farming

The concept

Better management practices (BMPs) are innovative, dynamic, and improved farming practices applied to shrimp farming and production systems to help ensure that sustainable development is achieved in an environmentally responsible manner. BMPs protect wildlife and coastal ecosystem as it primarily works to develop vitally needed quality shrimp production lowering the risk of disease outbreak and assuring sustainability, food security and safety.

The Origin: Aquaclubs adoption of BMPs

As a part of the technical collaboration between NACA and MPEDA and on shrimp desease control in India, village demonstration programmes were conducted during the year of 2003, 2004 and 2005. These demonstration programme were successful in organizing small-scale farmers into self-help groups (Aquaclubs) for adoption of "Better Management Practices" formulated in 2000-2002 period. The objective of that exercise was to produce better quality of shrimps reducing disease outbreak in socially acceptable, environmentally sound and economically viable manner through organization of "Self Help Groups"/ Aquaclubs distributed in clusters. Aquaclubs were linked to farm input suppliers with Contracts Systems for various benefits, mainly quality assurance.

CIBA's standpoint and BMP: In principle, the BMP reflect the guidelines framed by CIBA for shrimp farming, also these are close to that laid by the Aquaculture authority. Restricted or no use of artificial chemical fertilizers and pesticides, chemotherapeutic medicines including antibiotics is encouraged thus giving emphasis on utilization of natural nutrients, probiotics and bioremedial measures.

The overall purpose of our involvement is to provide scientifically sound information to improve the design, selection and performance of BMPs. BMP database project featuring more technical documents, software and database can be developed and with more scientific basis. The BMP developed over the past six years can be strengthened and amended with regard to different farming systems. What we need to look in to includes standardized BMP monitoring and reporting protocols, a storm water BMP database, BMP performance evaluation protocols.

Farming systems and BMPs: The traditional, extensive, semi-intensive and intensive farming systems are the major systems of shrimp farming. However, evolving culture practices like zero water exchange biosecured system and organic farming are in place time to time to cope with the situations. CIBA have initiated the organic way of farming has to be popularized in this region and elsewhere so that it can reverse the depleting productivity, biodiversity, mangrove and other habitat in this region. It is evident that the manner in which the BMPs influence the performance varies depending on the farming system, its state, level of adoption.

What are Best Management Practices?

The continued support and use of BMPs will help to ensure a sustainable shrimp production program that is conducted in a manner that minimizes harm to the environment while serving the sustenance of coastal aquaculture industry.

BMP Category	No of steps
Good pond preparation	15 each
Quality seed selection	
Water quality management	
Shrimp health management/biosecurity	
Feed management	
Pond bottom monitoring	
Emergency disease management	
Harvest and Postharvest management	
Mangrove plantation and conservation	
Environmental awareness	

Impacts of BMP includes Social Environment, Reduced costs and improved Profits, Reduced risk to small-scale farmers, Increased co-operation and harmony among farmers , Better organized farmer groups, Reduced disease incidence, Reduced FCR and increased efficiency of resource use (feed, seed, energy, finance in particular), Reduced pollution ,

Reduced chemical and antibiotic use. Following are some important BMP followed in shrimp farming:-

Good Pond Preparation: Good pond preparation is key to reducing disease risks and improving shrimp production. BMP for it includes removing the waste black soil away from pond manually labor or with machines after drying, removing the bottom algae if any, ploughing the pond bottom when wet or geting the pond bottom wet for at least 3 days before ploughing, repeated ploughing (if required), level of lime application depending on soil pH, Reservoir for every couple of ponds, proper screening by two layers of fine nets (60 mesh per inch) in the inlet, Ten days before stocking fertilization with organic/ inorganic fertilizers, >80 cm of water depth all time

Quality seed selection: Organized stocking within specific time period in the same locality and same batch in adjacent ponds; proximity of hatchery, uniform size seed and PL (12 mm or above); light gray or brown in colour (as signs of red or pink coloration are normally related to stress), Healthy fry swim straight against the current-should not concentrate in the bottom when stired; Salinity stress test; Microscopic test; (muscle:gut thickness 4:1); negative for MBV and WSSV. the transportation bags (2 liters of water) are 1000 (PL20) to 150 (PL15) maximum. Transport during morning or evening time, eliminating weak PL; 100 ppm formalin (50ml/ 500litres) and Ideal stocking in nursery should be 100 PL/cu m. Contract hatchery seed production system-45-60 days in advance of the planned stocking date help ensure better quality seed and bargaining capacity.

Water Quality Management: Water quality has a great influence on the efficiency of shrimp production. Fertilisation with organic (10-30 kg./ha.) and inorganic fertilizers (1-3 kg./ha.) to get bloom during first 6 weeks to help maintain the natural productivity, growth of benthic algae can be avoided by maintaining more water depth; benthic or floating algae in the pond- manually best; change 5-10 cm water and add lime (100 to 200 kg Agri. lime per ha); ideally water exchange should be 10% each time after 7 days retention in reservoir prior use; no water exchange till next tide if nearby disease affected pond-; If water colour is too dark- feeding is to be stopped during this time, agrilime for reducing the fluctuation also after heavy rain/ exchange, applying quick lime (CaO) to avoid acid soil or orange water; Aeration is required after 30-40 days of culture during late evening to early morning period in ponds with >5 pcs per meter density, cross transfer equipments between the ponds should be prevented.

Better Feed management: Cost of feed accounts for about 40% to 50% of the total production cost. BMP for feed includes starter feed with sprinkle water is fed 2 to 4 meter area from the edge, A mix of two feed pellet sizes for at least 7-10 days if there is any size variation; Active swimming of shrimp around the edge of the pond during daylight indicate

feed shortage; checking fullness of the gut 2 hours after feeding, If not increase the feeding rate; tray monitoring and demand feeding 30 DOC onwards; Shifting feeding area at least once in 7 to10 days depending on the bottom condition along feeding area. This allows shrimps to feed in a clean area. Feeding in pond corners and areas where it is dirty (black) must be avoided. Feed in the areas cleaned by the water movement by aeration. It is preferable to switch off the aerators just before feeding until the feed trays are checked (1-3 hrs). Reduce feeding during periods of low DO, plankton crash, rain fall, extremes of temperature never over feed. Slightly under feeding is better than over feeding, which saves money and reduce disease risks and during disease outbreaks. Proper storage

Pond Bottom Management: As the crop progresses, the bottom condition deteriorates depending on the stocking density and feeding practice. Soil color is black and smells bad, try to spread the feed further away from the dike (middle feeding), black soil occurs should be mildly and carefully agitated to dislodge the soil from the pond bottom during water exchange; benthic algae and Hydrilla can be prevented –for better pond bottom; chain dragging in one forth of the pond; pond corners cleaning; after harvest black sludge removal; plantation and grass turfing on pond dyke

Shrimp Health Management: The most successful strategies for controlling diseases in shrimp ponds are based on a combination of prevention by exclusion, and BMPs that focus on creating a healthy, non-stressful environment for the shrimp.Sick or dead shrimp-oxygen water quality –an indication; check gills, gut content, water quality and pond bottom condition, In case of wsd-informing neighbours; mortality increasing over 2 days don't change the water, in case. If >50% of the shrimp are not feeding, harvesting can be considered without draining the pond.

The gut content colour is a good indicator of the probable health status and corrective action to be taken. A black/ brown/ green gut content implies under feeding whereas a red or pink gut showed disease manifestation whereas a pale whitish gut showed gut infection. A normal gut will have a light or golden brown colour. Adding lime to the water (100-200 kg CaO/ha) and spread lime on pond dikes if after rain shrimps distress immediately not feeding shrimp with crustaceans (crabs or shrimp) or by catch waste, following BMP not feed shrimp with crustaceans (crabs or shrimp) or by catch waste.

Better Practices for emergency harvesting: Emergency harvest prevents spread of the disease to neighboring ponds and preserves the freshness and quality of the harvested shrimp; If daily mortality remains low (<5) or subsides-no harvest & water exchange is required but informing to neighbors is mandatory; If shrimp size are small, do not abandon/ drain-consider disinfecting the ponds; flag system; Separate any dead, discolored shrimp; Chill killing; Closely monitor neighboring ponds for shrimp health

Better Practices for harvesting and post harvest handling: Pond bottom should be cleaned be with out any dirty area, & exchange is done in case of heavy bloom, avoid harvesting during molting newly molted shrimps are >10%, delay the harvest by a day or two, Three to four days before harvest applying Agri. lime (100-200 kg/ha); 6 hours prior to harvesting –no feeding; completeing harvesting process in 6-8 hrs. Harvesting between 6 PM to 6 AM to avoid hot time ; avoiding using cast nets for harvesting; dip the harvested shrimps in slurry of ice for not less than 15 minutes are some important considerations. If possible use fresh water to make this ice slurry, which weight by 5%; transport crates with crushed ice at 1:1 ratio for better preservation; cleanliness all time.

Mangrove plantation and conservation: Mangrove trees are the best buffers against winds and waves, Mangrove trees (root, leaf and stem extracts of *Rhizophora)* have many medicinal properties. They are found to inhibit human pathogenic organisms; Mangrove saplings could be easily grown in the nurseries with the locally available seeds/ wildlings. No mangrove deforastration for shrimp pond construction and conserving the existing mangroves are for the BMPs.

Conclusion: Widespread adoption of better management practices in the shrimp farming sector, leading to improved yield, and a safe, quality and environmentally sound shrimp product for domestic and international shrimp markets. In brief the best management practices includes soil black layer removal, water screening with filter bags, two step PCR screening of the seeds, "All in All out"- one time stocking, on farm nursery, restriction on chemicals, antibiotics, demand feeding using check tray, safe disposal of dead/ diseased shrimps, black soil, benthic algae and smell check in bottom and its removal, emergency harvesting in case required, proper harvesting and mangrove restoration. System specific and cost-effective, better management practices (BMPs) incorporating principles of eco-based management including biosecurity should be developed, demonstrated and validated further to make the shrimp farming sustainable.

Guidance for monitoring these protocols for healthy shrimp farming includes

- data entry software to store and report BMP monitoring study data;
- performance summaries for individual BMPs
- Farming system specific modifications required in BMPs
- Analysis and evaluations of BMP performance
- statistical summaries of the overall BMP database

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TAXONOMY OF CULTIVABLE BRACKISH WATER SHELL FISHES

P.S. Shyne Anand, A.Panigrahi and G.Biswas

Introduction

Cultivable brackish water or marine shrimps are belonging to the largest phylum in the animal kingdom, the arthropoda, characterized by jointed appendages and an exoskeleton or cuticle that is periodically molted. Penaeid shrimp belong to genus *Penaeus* are the prime targets of capture fisheries and the favored species for aquaculture. Most widely cultured and economically important penaeid shrimps in India are *Penaeus monodon*, *P.indicus*, *P.merguiensis* and *P japonicus* Penaeid species are mostly easy to identify and many have distinctive colouring, which in the adults is fairly consistent (e.g. "tiger" prawns).

Classification: Penaeid are classified as

Phylum: Arthropoda (joint legged animals)

Class: Crustacea (shelled animals)

Sub Class: Malacostraca

Superorder: Eucarida

Order: Decapoda (ten foot animals)

Suborder: Natantia (swimming decapods)

Superfamily: Penaeoidea

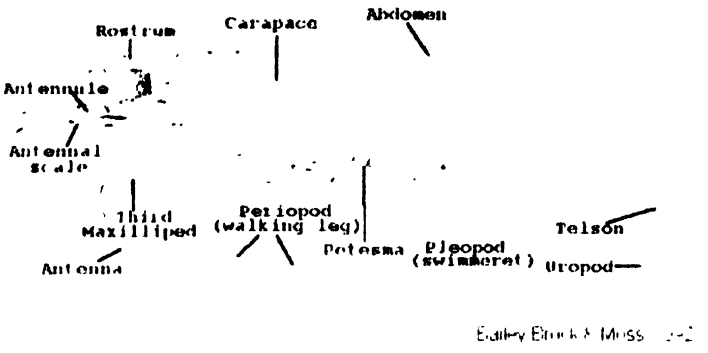
Family: Penaeidae

Genus: *Penaeus*

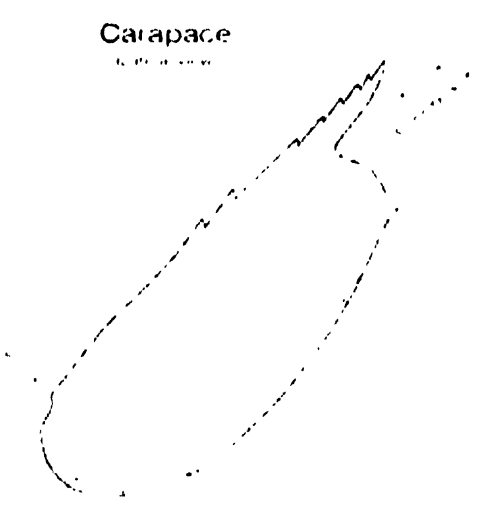
Species: *monodon, japonicus, indicus, merguiensis, chinensis*

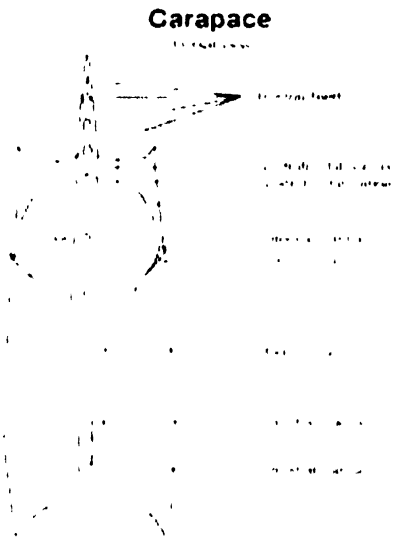
Adult penaeid shrimp, follow the general malacostracan plan. They are laterally compressed, elongate decapods, with a well-developed abdomen adapted for swimming. Each somite (segment) is enclosed by a dorsal tergum and ventral sternum, The side plates or an extension of each somite is known as pleura. They belong to super order eucarida as they have indirect development (i.e. one or more independent larval stages), no brood pouch (developing eggs are usually attached to appendages), and a distinct carapace.

Body segments: In Penaeidae, the head (five somites) and thorax (eight somites) are fused into a cephalothorax, which is completely covered by the carapace. The pleura of the cephalothorax form the branchiostegite or gill cover. The carapace has characteristic ridges (carinae) and grooves (sulci). The rostrum is always prominent, with dorsal and ventral teeth as well. The compound eyes are stalked and laterally mobile and the somites of the cephalic region bear, 5 pair of appendages i.e. pairs of antennules, antennae, mandibles, maxillules (maxillae 1) and maxillae (maxillae 2). The thorax has 8 pair of appendages i.e. three pairs of maxillipeds and five pairs of pereopods (legs), the first three being chelate and last two are simple (non-chelate). The abdomen consists of six somites, the first five with paired pleopods (swimming legs) and the sixth with uropods. The mouth is situated ventrally and the cephalic appendages and three maxillipeds surrounding it collectively known as the mouth parts. The anus is on the ventral surface of the telson towards its base.



Rostrum: Rostrum toothed both dorsally and ventrally, length variable, adrostral carina extending well back on to the carapace. Cervical and orbito-antennal sulci, antennal carina, hepatic and antennal spines are well defined; pterygostomial angle rounded. Dorsal carina is

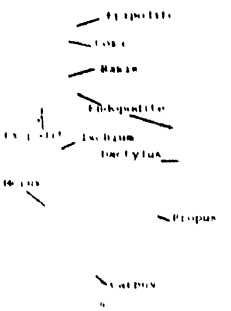




generally seen on the 4th-6th abdominal somites; Telson with deep median sulcus, without subapical fixed spines, with or without lateral movable spines. Antennular flagellum is

shorter than the carapace. Rostral grooves and ridges are one of the most important taxonomical tools for identification.

The endopodites of the walking legs are attached to the cephalothorax by a short joint, the coxa. Each appendage is consisting of a number of articulating sections called the basis, ischium, merus, carpus and propodus and the dactylus. The presence of a spine or row of spines on the ischium is of taxonomic importance for some species.



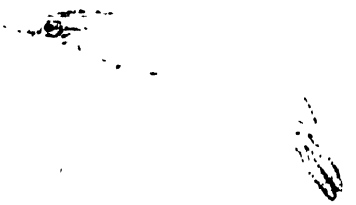
A typical appendage: Petasma in male is pod-like and flexible with thin median lobes. Thelycum in female is formed by the modification of strenal plate between the coxae of the 4th to 6th pereopods; Seminal receptacle occupying the ventral surface of the last thoracic somite, usually closed by two flaps, or sometimes a single pocket, or sometimes open. Petasma and thelycum are also used as one of the important species specific characteristics.

Identification characters of cultivable brackish water penaeid shrimps

***1.Penaeus monodon* (Fabricus, 1978)-Giant tiger shrimp:** Rostrum has 7-8 dorsal teeth and 3-4 ventral teeth and curves down very slightly. Rostral ridge lacks a distinct groove behind it, and the hepatic ridge is long and curved. Telson has a groove but is without lateral spines.

Color: Carapace and abdomen have black bands giving a tiger-striped appearance to this species. Pereiopods may be red.

2. *Penaeus indicus* (Milne Edwards, 1837) - Indian white prawn: Rostral crest elevated with 7-9 dorsal teeth and 4-5 ventral teeth. Adostral groove is distinct, but close to the median groove and reaches almost to the middle of the carapace.



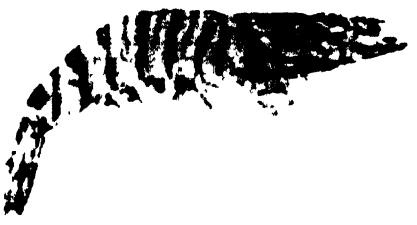
Gastro-orbital ridge is well defined and hepatic ridge is absent. Telson is broadly triangular and has a median longitudinal groove which lacks spines.

Color: overall creamy white small specks of blue, legs may be red and the rostral region brown.

3. *Penaeus merguiensis* (de Man, 1888) - Banana prawn: Rostrum extends horizontally and has an elevted crest with 6-10 large teeth dorsally and up to 6 ventral teeth. Median and adostral grooves are shallow and diminish at the middle of the carapace. Gastro-orbital ridge is absent or weakly defined and there is no hepatic ridge so the carapace appears smooth.

Color: overall creamy white.

4. *Penaeus japonicus* (Bate, 1888) - Kuruma prawn: Smooth, shiny carapace without hairs. Rostrum is almost horizontal but curves down very slightly, and has 8-10 dorsal teeth and usually a single ventral tooth. Adostral and median grooves reach the posterior margin of the carapace. Adostral groove is narrower than the postrostral ridge. Telson has moveable lateral spines. Color: brown bands (usually 10 or more) are especially conspicuous on the abdomen, but also apparent on the dorsal part of the carapace, legs and uropods. Legs are red and the telson and uropods are tinged with red, blue and yellow



Identification characters of cultivable brackish water mud crabs: Edible mud crabs belonging to the order brachyura and family portunidae. Two mud crab species, *Scylla tranqubarica* and *S. serrata* are commonly used in brackish water aquaculture.

Classification:

Phylum: Arthropoda (joint legged animals)

Class: Crustacea (shelled animals)

Sub Class: Malacostraca

Superorder: Eucarida

Order: Decapoda(ten foot animals)

Suborder: Reptantia

Infra order: Brachyura

Genus: *Scylla*

Species: *tranqubarica, serrata*

Brachyurans are true crabs; abdomen is thin, bent under cephalothorax, no uropods. 1st pair of walking appendage is in the form of heavy chelipeds. Pleopods are reduced in

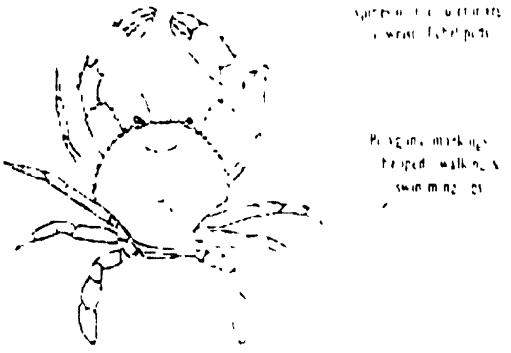
number and seen under abdominal flaps. Last pair of walking leg is flat in shape and modified for swimming.

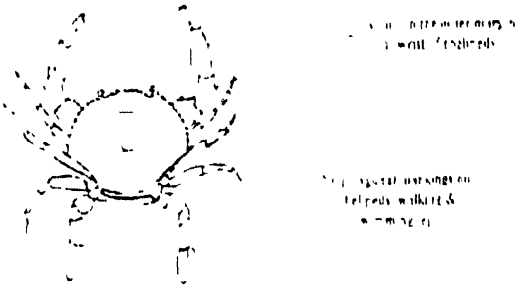
***Scylla tranqubarica* (Fabricus) - Green mud crab**

1. Polygonal markings on the walking leg and swimming legs
2. Spines on the outer margin of the wrist of the chelipds.

***Scylla serrata* (Forsklal) - Red mud crab or mangrove crab**

1. No Polygonal markings on the walking leg and swimming legs.
2. One pines on the outer margin of the wrist of the chelipds.





Scylla tranqubarica *Scylla serrata*

Suggested Readings:

Baily-Brook, J. H. and Moss, S. M. (1992). Penaeid taxonomy, biology and zoogeography; in Marine Shrimp Culture: Principles and Practices, Fast, A. W. and Lester, L. J. (eds.), pp. 9-27, Elsevier Science Publishers, Amsterdam, Netherlands.

FAO Species identification sheet.

Kathirvel, M.; Kulasekarapandain, S; Balasubramanian, C.P (2004) .Mud crab culture in India, CIBA bulletin No: 17.

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TAXONOMY AND IDENTIFICATION OF CULTIVABLE BRACKISHWATER FINFIHSES

G. Biswas

Taxonomy is the theory and practice of classifying organisms. Although sophisticated cytogenetic or biotechnological techniques are presently available to find out differences among individual species, at field level still a species is identified based on some morphological characteristics. Here, taxonomy and key characters for identification of commercially important brackishwater fishes are discussed.

1. Asian seabass

1.1 Taxonomy

Phylum : Chordata
Class : Osteichthys
Order : Perciformes
Family : Centropomidae
Genus : *Lates*
Species : *Lates calcarifer* (Bloch)

1.2 Key characters for identification

- Mouth large, slightly oblique, upper jaw reaching to behind eye, teeth villiform.
- Lower edge of pre-operculum with a strong spine, operculum with a small spine and with a serrated flap above origin of lateral line.
- Dorsal fin with 7-9 spines, anal with 3 spines, both dorsal and anal have scaly sheaths.
- Scales ctenoid.

2. Milkfish

2.1 Taxonomy

Phylum : Chordata
Class : Osteichthys
Order : Gonorhychiformes
Family : Chanidae
Genus : *Chanos*
Species : *Chanos chanos* (Forsskal)

2.2 Key characters for identification

- Body elongate, moderately compressed with no belly scutes.
- Mouth small, transverse, without teeth, upper jaw slightly projecting, lower jaw with a small symphysial tubercle at tip, fitting into a notch.
- Dorsal fin at mid point of body, anal fin short close to caudal, pectoral and pelvic with large auxiliary scales, caudal deeply forked.
- Body olive green, sides silvery.

3. Striped grey mullet

3.1 Taxonomy

Phylum : Chordata

Class : Osteichthys

Order : Perciformes

Family : Mugilidae

Genus : *Mugil*

Species : *Mugil cephalus* Linnaeus

3.2 Key characters for identification

- Head broad and flattened on top, its length 27-29% of standard length.
- Fatty (adipose) tissue covering most of eye.
- Posterior tip of upper jaw not curved down and hidden when mouth closed.
- Origin of second dorsal fin behind vertical from origin of anal fin.
- Pectoral fins short with auxiliary scales, broad dark blue notch at pectoral base.
- Body olive green on back, silvery on sides, 6-7 indistinct longitudinal brown bars on flanks.

4. Tade grey mullet

4.1 Taxonomy

Phylum : Chordata

Class : Osteichthys

Order : Perciformes

Family : Mugilidae

Genus : *Liza*

Species : *Liza tade* (Forsskal)

4.2 Key characters for identification

- Head short, broad and flattened on top, its length 19-23% of standard length.

- Fatty (adipose) tissue covering eye except for pupil.
- Posterior tip of upper jaw strongly curved and still visible when mouth closed.
- Pectoral very short with auxiliary scale very small or absent.
- Body with olive back, flanks and belly silvery, often 5-9 dark longitudinal stripes along flanks.

5. Goldspot mullet

5.1 Taxonomy

Phylum	Chordata
Class	Osteichthys
Order	Perciformes
Family	Mugilidae
Genus	*Liza*
Species	*Liza parsia* (Hamilton & Buchanan)

5.2 Key characters for identification

- Body slender, head moderately wide, dorsally flattened, head 23-26% of standard length.
- Fatty (adipose) tissue covers most of iris posteriorly and part of it anteriorly.
- Pectoral auxiliary scales absent.
- Body colour greenish brown above, white to silvery below, a golden spot on upper operculum, base of second dorsal, anal and caudal fins yellowish.

6. Pearlspot

6.1 Taxonomy

Phylum	: Chordata
Class	: Osteichthys
Order	: Perciformes
Family	: Cichlidae
Genus	: *Etroplus*
Species	: *Etroplus suratensis* (Bloch)

6.2 Key characters for identification

- Body elevated and laterally compressed. Cleft of mouth is small.
- Single dorsal with 18-19 spines, spinous part much longer than soft part.
- Body light green with 6-8 vertical bands.
- Scales above the lateral line with a central pearly spot.

7. Spotted scat

7.1 Taxonomy

Phylum : Chordata

Class : Osteichthys

Order : Perciformes

Family : Scatophagidae

Genus : *Scatophagus*

Species : *Scatophagus argus* (Bloch)

7.2 Key characters for identification

- Body quadrangular, strongly compressed.
- Forehead steep, mouth small with brush-like teeth.
- Dorsal fin with 11 spines, the membranes deeply incised between spines, middle of dorsal fin with a deep notch.
- Body colour greenish to silvery with numerous dark spots mainly confined to upper portion of sides.

Suggested Reading:

Inland Fishes of India and Adjacent Countries by P. K. Talwar and A. G. Jhingran. Oxford and IBH Publishing Co., New Delhi.

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ISOLATION OF PATHOGENIC BACTERIA FROM FINFISH AND SHELLFISH

Sujeet Kumar and R. Ananda Raja

Introduction

The first step in diagnostic bacteriology is to isolate the pathogen from the diseased animals as pure culture. For comparative purpose, bacteria may be isolated from infected and apparently healthy specimen at a time. As putrefying bacteria act upon the dead fish or shell fish very quickly, isolation of bacteria should be carried out from the just dead or moribund fish. Final identification is made based on its cultural, morphological, physiological, biochemical, serological and molecular characteristics.

Aseptic techniques: Maintenance of aseptic conditions during all steps of microbiology is the first step for successful microbiological investigation. For ensuring this, all glassware, plasticware, media, solutions etc are sterilized before use. Personnel care should be taken to wash the hands with 70% ethanol before start of any culture work. Sterilization methods employed for different kinds of materials used in the laboratory are given in Table 1.

Table.1 Sterilization methods for common labware

No.	Materials	Methods of sterilisation
1	All types of glassware like pipettes, tubes, flasks, petridish etc. and lipid like paraffin oil	Dry heat Hot air oven at 160^{0}C for two hour or 180^{0}C for one hour.
2	Bacteriological media, discarded media, plasticware, steel items, corks, rubber materials, filter pads, distilled water, buffers, solutions and also glassware	Moist heat Autoclaving at 121^{0}C, 15 lb pressure for 15 min.
3	Tissue culture media, antibiotics, sera, heat sensitive solutions like carbohydrate, aminoacids etc.	Filtration by 0.22µm or 0.45 µm pore size membrane filter.

Isolation of pure culture of bacteria: Isolation of pure culture of bacteria is necessary to characterize it further by cultural, biochemical, molecular methods. A number of methods are used for this purpose. These are:

1. Streak plate technique (streaking onto solid media)
2. Pour plate technique (Incorporation into molten semi-solid media)

3. Dilution in liquid media

Streak plate method: The streak plate method is most commonly used for obtaining pure culture of specific bacterium from mixed bacterial populations.

Procedure

1. Inoculate the infected larvae/ affected tissues/ haemolymph/water sample on the culture plates with the help of sterile bacteriological loop and streak the inoculum (fig.1) to get isolated colonies.
2. Incubate the inoculated agar plates at optimal temperature (28^0 - 30^0C) for 24-48 h and observe for development of bacterial colonies.
3. Examine cultural characteristics of the bacterial colonies as given in the subsequent sections and record.
4. Obtain pure culture of bacteria by picking up morphologically distinct colonies with the help of a sterile bacteriological loop and subculture on ZMA for further characterization.

Fig.1 Inoculation of petridish by streak plate method.

Identification of bacteria: For identification of bacteria, cultural characteristics like size, shape, pigmentation, opacity of the bacterial colonies on the solid media and morphological characteristics like cell shape (rod, cocci, coccobacilli, comma), sporulation, flagellation, capsule etc. are important and aid in preliminary grouping of the bacteria. Some of the cultural and morphological characteristics useful for distinguishing bacteria are presented in Tables 2 and 3. Additionally, some routine tests such as Gram's staining, motility, oxidase, catalase, oxidation-fermentation test etc. are routinely conducted to identify bacteria upto genus level. These routine bacteriological tests are presented in table 4.

Table 2. Cultural characteristics of bacteria grown on solid media

Sl. No.	Colony character	Description
1	Shape	Circular, irregular, radiated, rhizoidal etc.
2	Size	Size of colony in mm
3	Surface	Smooth, contoured, rough, ridged, striated, dull, glistening
4	Edge	Entire, undulate, lolate, crenated, fimbriate, effuse, spreading
5	Opacity	Translucent, transparent, opaque
6	Colour	Different colours due to production of pigments

Table 3. Morphological characteristics of bacteria

Sl. No.	parameter	Description
	Shape	Cocci, oval, short rod, long rod, filamentous, comma, spiral
2	Size	Lengtth and breadth in µm
3	Arrangement	Single, pairs, chains, in four (tetrads), in groups, grape like clusters, irregular
4	Flagella	Polar, monotrichous, amphitrichous, peritrichous
5	Spores	Spherical, oval, elliptical, single or multiple, terminal, subterminal or central
6	Capsule	Present or absent
7	Staining	Gram positive or negative

Table 4: Routine bacteriological test for identification of bacteria

Sl. No.	Test	Basis	Inference
1	Gram's staining	Cell wall peptidoglycan helps in retaining dye	Gram + bacteria show violet colour and Gram – bacteria Red/pink colour
2	Motility test (Hanging drop method	Presence of flagella	Zig-zag motility indicates presence of polar flagella
3	Oxidase test	Presence of cytochrome oxidase	Development of blue colour shows positive reaction

4	Catalase test	Catalase enzyme which breakdown toxic hydrogen peroxide	Production of gas bubble (Effervescence) indicate positive reaction
5	Carbohydrate fermentation test	Utilisation of sugar in presence and absence of oxygen	Acid production in open tube indicate oxidative metabolism while in paraffin covered tube fermentative metabolism
6	Indole test	Production of indole by degradation of tryptophan	Development of pink colour indicates positive reaction
7	Voges proscauer's test	Detect acetoin or acetyl methyl carbinol an intermediate product of glucose metabolism	Production of red/crimson colur indicates production of acetoin
8	Sensitivity to O/129	Vibrio are sensitive to O/129	Differentiate *Vibrio* from closely related bacteria like *Pseudomonas*. *Aeromonas* etc

Suggested Readings:

Schneider, J., and G. Rheinheimer (1988). Isolation methods. In: Austin, B. (Eds). Methods in Aquatic Bacteriology: pp 73-94.

Surendran, P.K., N. Thampuran, Nambiar, V.N. and K.V. Lalitha (2006). Laboratory manual on microbiological examination of seafood. Central Institute of Fisheries Technology (Publisher) pp: 1-43.

Dart, R.K. (1996). Microbiology for the analytical chemist. Pp 25-55.

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IDENTIFICATION OF DIFFERENT FEED INGREDIENTS USED IN AQUAFEED AND QUALITY ASSESSMENT

Debasis De and T.K.Ghoshal

Major ingredients generally used for formulation of aquafeed are wheat flour, rice flour, maize flour, soybean cake, ground nut cake, cotton seed cake, sun flower cake, fish meal, prawn meal, prawn head meal, squilla, squid, clam meal, cuttle fish, meat meal, silk worm pupae meal, shark liver oil, cod liver oil, fish oil, soybean oil, soyalecithin, sunflower oil, safflower oil, brewer's yeast, spirulina, mineral mixture, vitamin supplement and binder (guar gum, cellulose, hemicellulose and synthetic binder).

Controlling the quality of feed ingredients is and essential prerequisite for the success of any Livestock farm. Since feed cost contributes 60 to 70% of the cost of aquaculture production the importance of quality control of feed ingredients plays a major role.

Quality is the sum of the characteristics of a product of service that have a bearing on its ability to satisfy a customer's need, stated or implied, limited by the price and delivery time, he or she will accept. In order to assure the quality of complete feed, it is essential to specify the minimum acceptable quality standards of feed ingredients.

Sources of Quality standards for Feed Ingredients: There are several sources/agencies from which we can obtain the specifications for various ingredients. Some of these sources are listed below:

i) BIS ii) NRC iii) USDA etc.,

Each ingredient can be tested for dozens items like

i) Proximate principle ii) Minerals iii) Fatty acids iv) Amino Acids v) Vitamins

vi) Pesticides vii) Mycotoxins viii) Infectious agents ix) Adulterants x) Toxicants, etc.,

Obviously it is not possible to test all the ingredients for all the parameters mentioned above, since the cost and time involved are prohibitive. Therefore, the parameters should be selected based on the need/importance of the parameter involved. The cost of testing and time required for testing will limit the number of tests conducted. Test should be selected based on (a) Need (b) Cost (c) Time. Specifications for each ingredient should be Comprehensive, Realistic, Transparent and in writing.

Some examples for specifications are shown in Table - 1.

Table – 1: Specifications for some common Ingredients

Ingredient	Ideal	Accept	Reject
Maize			
Moisture	<10.0%	10.5%	>12.0%
Aflatoxin B1	<0.05 ppm	Upto 0.1 ppm	>0.1 ppm
Soybean Deoiled Cake			
Protein	>47.0%	46.0%	<45.0%
Di-Calcium Phosphate			
Phosphorous	>18.0%	17.0%	<16.0%
Flourine	<0.1%	0.1%	>0.1%

Please note that these specifications may be varied from plant to plant depending on the price, season, ingredient availability, willingness to take risk etc.,

Tests normally conducted in Feed ingredients: Some of the tests normally conducted on feed ingredients are listed below. It should be noted that individual plant/company may add or delete some of the tests depending on its need.

1. Grains Moisture, Mycotoxins, Thyram
2. Oil meals Moisture, Protein, fibre
3. Rice Bran Moisture, Fat, Fibre, Sand and Silica
4. DORB Moisture, Protein, Fibre, AIA, Mycotoxins
5. Fish Meal Moisture, Protein, Sand and Silica, Salt
6. Meat Meal Protein, Fat, Ca, Phos, Sand and Silica
7. DCP Moisture, Calcium, Phosphorous, Flourine
8. LSP Calcium, Mg

Classification of Tests Conducted on feed ingredients: The tests conducted on raw materials can be classified as follows.

a) Physical

(1) Visual, (2) Touch, (3) Smell, (4) Taste, (5) Soaking in Water

b) Chemical

Wet Chemistry

c) Biological

(1) Microbial Contamination

d) Feed Microscopy

e) Non Destructure

Near Infra Red Spectroscopy Techniques (NIR)

Visual Examination

1. Colour Consistency, 2. Grain Size, 3. Cake formation, 4.Mould Growth, 5.Adulteration with foreign materials, 6.Over/Under toasting of SBM, and 7.Shells, crabs etc. in fish.

Touch

Put hand deep into bag. Temperature indicated moisture level.

Taste

1. Freshness of Cakes, Bran, 2.Salt level in fish, 3.Bitterness indicated mycotoxins, 4.Coarse Sand, and 5.Moisture level in grain.

Smell

1. Freshness/Rancidity, 2.Musty odour – Mycotoxins & Mould growth, and 3.Fermentation due to high moisture.

Soaking

1. Fiber level, and 2. Sand Content.

Feed Microscopy

1. Needs extensive training, 2.Detects adulterants, and 3. Can identify most of the ingredients in a mixed feed.

There are some rapid tests which can be conducted while the truck is waiting.

1) Electronic moisture meters for grains

2) Cresol Red test for under/over toasting of SBM

3) Urea adulteration is fish meal/oil meals

4) Ultraviolet lamp blue green fluorescence indicates mycotoxins

These tests have some advantages and some disadvantages as indicated below.

Advantages of Rapid Tests

1. Inexpensive, rapid, simple
2. Can be done while truck is waiting
3. First step before more formal testing

Disadvantages

1. Quantification is difficult
2. May not be acceptable in legal cases

COMMONLY USED INGREDIENTS AND THEIR SALIENT FEATURES

Feed Ingredient	Checks to be made for	Common Adulterants	Mycotoxin/ANFs Occurrence
Maize	Freshness, Colour, Size, Moisture, Heat, Mouldy odor, Weevils Pesticide – Thiram Bulk density 0.725 to 0.775 kg/litre	Cobs and Cob dust, Sand	Aflatoxin, Citrinin Cyclopizoniz Acid ochratoxin
Bajra	Freshness, Colour, Size, Moisture, Heat, Weeds, Sand and Silica Pesticide – Thiram Bulk density 0.72 to 0.76 kg/litre	Certified seed contamination, Sand	T2-Toxin, Zeralenone NSPs
Rice	Freshness, Colour, Mouldy, Odour, Sand, Husk, Rancid odour Bulk density 0.70 to 0.775 kg/litre	Sand, Bran, Husk	Aflatoxins, Ochratoxins
Wheat	Freshness, Mouldy, Odour, Sand, Husk, Weed Seed Bulk density 0.70 to 0.77 kg/litre	Weed Seeds, Husk Sand	Aflatoxins, Ochratoxins, NSPs
Soybean	Freshness, Moisture, Clumps, Odour, Colour, Mould Growth Bulk density 0.52 to 0.57 kg/litre	Sand & Silica Hulls (fibre)	Aflatoxins, Trypsin inhibitors Emerging toxins, NSPs
Groundnut Cake	Freshness, Moisture, Colour, Heat, Odour, Clumps, Mould Growth Bulk density 0.65 to 0.70 kg/litre	Hulls (fibre) Sand, Other Cheaper oil seeds	Potential feed for Aflatoxins infestation, Ochratoxin
Sunflower Meal	Freshness, Moisture, Heat, Odour, Rancidity, Clumps, Mould Growth Bulk density 0.50 to 0.53 kg/litre	Hulls (fibre) Sand	Ochratoxin, Aflatoxin B1, T2-toxin, NSPs
Rapeseed	Moisture, Heat, Clumps,	Hulls (fibre) Sand	Aflatoxin B1,

Meal	Mould Growth Bulk density 0.65 to 0.775 kg/litre		Glucosinolates
Dry Fish/Fish meal	Moisture, Heat, Smell, Roughness, Clumps Bulk density 0.725 to 0.675 kg/litre	Sand,	Gizerosine
Rice Bran - Deoiled	Moisture, Heat, Smell, Roughness, Clumps Bulk density 0.35 to 0.40 kg/litre	Sand.	Aflatoxin
Ricepolish	Moisture, Rancidity, Coarseness, Oiliness, Odour Bulk density 0.4 to 0.42 kg/liter	Ricebran Husk, Saw dust, Sand	Aflatoxin
Calcite	Moisture, Colour, Coarseness	Sand, Magnesium	
DCP (Dicalcium phosphate)	Moisture, Colour, Odour	Sand, Fluorine	
Maineral Mixture	Moisture, Colour, Odour	Sand, Magnesium	
Meat and Bonemeal	Moisture, Odour, Colour	Sand, Leather meal	Biogenic amine: Microbiol contamination

Suggested Reading:

Ranjan. S.K. (1991) Chemical composition and nutritive value of Indian feeds and feeding of farm animals. ICAR publication

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DEMONSTRATION OF PCR AND RT-PCR

R. Ananda Raja and Sujeet Kumar

To get familiarize with the polymerase chain reaction (PCR), isolation and quantification of the genomic DNA from fish blood, RNA from muscle tissue and complete protocols used in PCR and Reverse Transcription Polymerase Chain Reaction (RT-PCR) are well demonstrated in this chapter with standardized methods.

Materials required

Buffers and solutions

1. Absolute ethanol.
2. Absolute Iso Propanol.
3. Agarose.
4. Anticoagulant: 0.5M EDTA.
5. Autoclaved Milli Q water.
6. Chloroform : Iso Amyl Alcohol - 24:1.
7. DNA Extraction Buffer (TEN): 100mM Tris-HCl (pH 8.0), 10mM EDTA, and 250mM NaCl.
8. DNA Loading Buffer: 0.25% bromophenol blue, 0.25% xylene cyanol FF and 15% Ficoll (Type 400) in water.
9. dNTPs (dATP, dCTP, dGTP, dTTP).
10. Ethidium bromide: 10mg/ml in distilled water as stock solution.
11. Fish blood and muscle samples.
12. Ladder- 100bp and 1kbp.
13. Methanol.
14. $MgCl_2$ for PCR
15. Normal slaine.
16. Oligo dT for RT-PCR.
17. PCR Buffer solution.
18. Proteinase-K : 20mg/ml in H_2O.
19. Reverse transcriptase for RT-PCR.
20. RNase inhibitor for RT-PCR.
21. Sodium acetate: 3M - pH-5.2.
22. Sodium dodecyl sulfate – 20%.
23. Specific primers for PCR.

24. *Taq* DNA Polymerase.

25. TBE buffer stock-5x: 54 g Tris base, 27.5 g Boric acid and 20ml of 0.5M EDTA pH adjusted to 8.0 per litre of buffer stock.

26. TE buffer: 10mM Tris-HCl (pH 8.0) and 10mM EDTA.

27. Tris saturated phenol - pH adjusted to 8.0 by using Tris.

All the chemicals used in this study are obtained from commercial sources and are of molecular biology grade.

Equipments

1. Adjustable pipettes, 2. Cold Centrifuge, 3. Electrophoresis apparatus, 4. Eppendorf tubes, 5. Glassware, 6. Homogenizer, 7. Shaking incubator, 8. Sphectrophotometer, 9. Thermal cycler, 10. Timer, 11. UV transillumunator, 12. Vortex mixer, and 13. Water bath.

Methods

Sterilized condition should be well maintained in all operations as a preliminary requisite.

I. Isolation of genomic DNA from blood samples by Proteinase K Method

Genomic DNA is extracted from blood samples by the standard Proteinase K digestion method (Sambrook *et al.*, 1989). 100µl of Methanol preserved fish blood sample is washed 6 times with normal saline followed by centrifugation at 5000 g for 5 min at room temperature. The cell pellet after the final spin is suspended in 400µl of TEN Buffer. Then, 20% SDS 20µl is added and mixed well. Proteinase K (final concentration 100µg/ml) is added and mixed thoroughly and incubated in water bath at 37°C for overnight. Equal volume of Tris-saturated phenol (pH 8.0) is added and homogenized followed by centrifugation at 12,000 g for 10 min. The upper aqueous layer containing DNA is collected using wide mouthed pipette tip. The aqueous layer is once again extracted with 100µl chloroform:isoamyl alcohol (24:1). To the aqueous phase, $1/10^{th}$ volume of 3M sodium acetate (pH 5.2) is added and mixed well. To this, 2.5 volumes of isopropanol is added and mixed gently. The DNA is formed as a visible precipitate and is pelleted at 12,000 g for 10 min. The pellet is washed twice with 70% ethanol. After air-drying (to remove traces of ethanol), DNA is dissolved in 100µl of TE buffer.

II. Quantitation and Quality checking of DNA

Spectrophtometric method

10µl of DNA is taken and diluted in 990µl of TE buffer (1:100 dilutions). The ratio of absorbance at 260nm and 280nm is a useful indication of quality of DNA. The ratio is 1.8 for

pure DNA. For the quantification of DNA, the O.D at 260nm is taken and the concentration of DNA is calculated as follows.

1 O.D of double stranded DNA at 260nm = 50µg/ml.

$$\text{Therefore DNA concentration } (\mu g / \mu l) = \frac{\text{O.D. X dilution factor X 50.}}{1000}$$

Agarose gel preparation and electrophoresis

A clean, dry and perspex gel-casting moult is sealed with adhesive tape on both sides and kept on a leveling platform after putting a comb on the slot. Agarose 1% is prepared in 1x TBE electrophoresis buffer with ethidium bromide to a final concentration of 0.5µg/ml and mixed thoroughly. Warm agarose solution is poured into the mold to the thickness 3mm to 5mm.There should be no air bubbles under or between the teeth of the comb. After the gel is set completely the comb and the tape are carefully removed and the gel is mounted in the electrophoresis tank filled with 1x electrophoresis buffer. The 3µl of DNA is mixed with the desired gel loading dye (Bromophenol Blue & Xylene cyanol) and glycerol mixture and slowly loaded into the wells of the submerged gel using a micropipette Electrical leads are connected to the respective electrode of the power pack. The voltage applied is 1-5V/cm distance between the electrodes. Electrophoresis is continued until the dye migrates to the appropriate distance in the gel. Gel from the tank is taken out and examined under UV light in a Transilluminator.

III. Isolation of RNA from muscle by Trizol method

Tissue sample is collected in Trizol reagent (1ml of Trizol per 50-100 mg of the tissue) and homogenized. The properly homogenized tissue sample is kept on ice for 5 min. and the pellet is removed by centrifuging 12,000 g for 10 min. at 4° C to remove membrane, polysaccharide and high molecular weight DNA. The supernatant contains RNA. To the supernatant, 0.2 ml of $CHCl_3$ per ml of Trizol reagent is added and kept on ice for 5 min. It is centrifuged for 12,000 g for 10 min. at 4° C which is separated into 3 layers-lower bulky mass, middle interphase and upper aqueous phase containing the RNA. The upper aqueous phase is then transferred to a fresh tube and isopropanol is added at the rate of 0.5 ml per every ml of Trizol used. It is again centrifuged for 12,000 g for 10 min. at 4° C to pellet the RNA. The supernatant is discarded and precipitated RNA is washed with 80% ethanol, dried, dissolved in nuclease free water and stored at -20°C.

IV. Quantitation and Quality checking of RNA

Spectrophtometric method

1µl of RNA is diluted in 99µl of nuclease free water. The ratio of absorbance at 260nm and 280nm is a useful indication of quality of RNA. For the quantification of RNA, the O.D at 260nm is taken and the concentration of RNA is calculated as follows.

1 O.D of double stranded RNA at 260nm = 40µg/ml.

$$\text{Therefore, RNA concentration } (\mu g / \mu l) = \frac{\text{O.D. X dilution factor X 40}}{1000}$$

Agarose gel electrophoresis is done as mentioned above with the fresh set of electrophoresis unit to check the quality of RNA.

V. Preparation of cDNA by RT-PCR Technique

In the RT-PCR technique the extracted RNA is then converted into complementary DNA strand with the help of Reverse Transcriptase enzyme and oligo dT primer which can then be used for the conventional PCR. One of the benefits of this technique is to identify rare and low levels of mRNA transcripts with greatest sensitivity. The reaction is done in the following way.

1. The following components are added in the microfuge tubes.

Total extracted RNA (1 µg /µl)	5.0µl (5 µg)
dNTP(10mM)	2.0µl (1 mM)
Oligo dT (0.5µg)	1.0 µl (0.5 µg)
Autoclaved Milli Q water	4.5 µl

Tubes are placed in a Thermal cycler and is heated at 65°C for 5min. (denaturation step) to remove all the secondary structures present in the RNA and immediately kept on ice for 5 min.

2. Then, the following components are added

RNAase inhibitor (40u/µl)	0.5µl (20 units)
Buffer (10x)	2.0µl (1x)
$MgCl_2$ (25mM)	4.0µl (5 mM)
Reverse Transcriptase (40u/µl)	1.0µl (40 units)

The reaction mixture is mixed gently and incubated in the Thermal cycler at 42° C for 1 hour followed by 85°C for 15 min. and then it is kept at 4° C forever.

VI. The Cycling Reaction

There are three major steps in a PCR, which are repeated for 30 or 40 cycles. This is done in an automated Thermal cycler, which can heat and cool the tubes with the reaction mixture in a very short time. For the total reaction volume of 25µl, the PCR tube is added with the following components.

Availability		Requirement / Reaction
1. Buffer without $MgCl_2$ 10x	-	2.5µl
2. $MgCl_2$ (25mM)	-	1.5µl
3. d NTPs (2.5mM)	-	2.0µl
4. Primer F	-	1.0µl (10pM)
5. Primer R	-	1.0µl (10pM)
6. DNA template (50-100ug)	-	1.0µl
7. Autoclaved Distilled Water	-	15.0µl

The PCR tubes are placed in the thermal cycler at 95° C for 5 minutes to remove all the secondary structures from DNA and immediately in ice for 5 minutes (initial denaturation). Then, 1.0µl (0.1-0.2U/µl) of the *Taq* Polymerase is added to the mixture and the thermal cycler is run as follows.

8. Denaturation - 94° C for 20 seconds.
9. Annealing - 55° C for 20 seconds.
10. Extension - 68° C for 90 seconds.
11. 35 cycles
12. Final Extension - 68° C for 10 minutes.
13. 4° C forever.
14. Program End.

The concentration of the ingredients, the temperature and time may be changed for every reaction to get the optimum PCR product amplification.

Suggested Reading:

Joseph Sambrook and David W. Russell (2001) Molecular cloning: a laboratory manual. 3rd Edn.: Cold Spring Harbor Laboratory Press, New York.

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ESTIMATION OF SOIL AND WATER QUALITY PARAMETERS

P.S. Shyne Anand, G.Biswas and A.Panigrahi

Introduction

Soil and water quality parameters are one of the most important factors that play a pivotal role in success of any aquaculture ponds. Before construction of the pond, soil quality parameters like pH, organic carbon content, available nitrogen and phosphorous to be checked to get an idea about the fertility status of the soil. During the production cycle, regular monitoring of water and soil quality parameters are essential to study the characteristics of pond soil and water. This help to mitigate the undesirable condition by adopting suitable managerial measures in culture systems. The most critical parameters to be checked regularly in culture ponds are pH, Dissolved oxygen, transparency, salinity, alkalinity etc.

Estimation of water quality parameters

Most of these parameters can be measured using various methods like potentiometry, conductivity, gravimetry, titrimetry and spectrophotometry.

1. PH: pH can be directly measured with a pH meter having a sensitive glass electrode based on potentiometric methods. Before estimation, the meter should be calibrated routinely appropriate buffer solution at pH 7.0 and a pH 9.

2. Salinity: The salinity of brackish water can be determined either using refractometer or by titrating the precipitable halides in water with silver nitrate solution using potassium chromate as indicator (Mohr methods).

3. Alkalinity: It can be measured by titrating the water sample with a standard acid using methyl orange. If the sample remains colourless while adding few drops of methyl orange indicator no alkalinity is there. If it is yellow, titrate with standard acids till the colour turns taint orange.

4. Turbidity: Turbidity can be caused either by planktonic organisms or by suspended soil particles. Turbidity due to suspended soil particles is measured by Nephelo-turbidity meter. Turbidity due to planktonic organisms i.e. transparency can be measured with the help of a Secchi disc.

5. Total settleable solids: Settleable organic and inorganic solids can be measured using an Imhoff cone by allowing the particles to settle in one hour and measure in ml/l.

6. Total suspended solids (TSS) and total dissolved solids (TDS) : It can measured using gravimetric methods by retaining the residue on a pre weighed filter paper and then dry to

103^0C -105^0C. An increase in weight of filter paper represents the total suspended solids. For total dissolved solids, the filtrate is evaporated to dryness in a weighed dish and dried to constant weight. The increase in dish weight represents the total dissolved solids.

7. Dissolved oxygen: DO can be determined by Winkler's method. In this method, a divalent manganese solution, followed by strong alkali i.e. winkler A and B respectively is added to the sample. Dissolved oxygen present in the water oxidises an equivalent amount of divalent manganese to basic hydroxides. When the solution is acidified in presence of iodide ions, the oxidised manganese ions again revert to divalent state and iodine, equivalent to the original dissolved oxygen content of the water, is liberated. This iodine is titrated with standardised thiosulphate solution.

8. Chemical oxygen demand: COD is a measure of organic matter and represents the amount of oxygen required to oxidize the organic matter by strong oxidizing chemicals (potassium dichromate) under acidic condition. The excess dichromate is titrated with standard ferrous ammonium sulphate using ferroin as an indicator. Mercuric sulphate is added to complex the chlorides, thereby effectively eliminating the chlorides interference.

9. Biochemical oxygen demand: The water sample after appropriate dilution is incubated for 5 days at 20°C in the dark. The reduction in DO concentration during the incubation period is measured using winkler method and it yields a measure of the BOD.

10. Ammonia-N: Water sample is treated in an alkaline citrate medium with sodium hypochlorite and phenol in the presence of sodium nitroprusside, which acts as a catalyser. The blue indophenol colour formed with ammonia is measured spectrophotometrically at 640 nm wavelength.

11. Nitrite-N: The nitrite in water is allowed to react with sulfanilamide in an acid solution. The resulting diazo compound formed is allowed to react with NED and forms a highly coloured azo dye. The colour formed is measured spectrophotometrically at 540 nm wavelength. Nitrite-N conc. is calculated using standard calibration graph.

12. Nitrate–N: Nitrate in water sample is reduced almost quantitatively using a reducing agent (copper sulphate solution and hydrazine sulphate) to nitrite. Then absorbance of the nitrite produced is determined spectrophotometrically at 540 nm.

13. Hardness: Calcium and magnesium ions present in water sample is titrated with the complexing agent ethylene diamine tetra acetic acid disodium salt (EDTA) to form the stable complexes. The end point of the titration is signaled with an indicator called Erichrom black-T.

Estimation of Soil quality parameters

1. pH: Potentiometric method with electrically or battery operated pH meter having suitable electrodes is used for determination of soil pH values.

2. Electrical conductivity: Electrical conductivity (E.C) is commonly used for indicating the total concentration of the ionized constituents of solutions in soil. It can be measured using a conductivity meter.

3. Organic matter: Organic matter content of the soil can be measured by digesting a known quantity of soil with known excess of chromic acid using the heat of dilution of sulphuric acid. The excess chromic acid, which is not utilized for the oxidation of organic carbon, is back titrated against standard ferrous ammonium sulphate solution using diphenylamine indicator till the bright blue colour changes to light green colour.

4. Available Nitrogen: A Known weight of soil is mixed with excess of alkaline potassium permanganate and distilled. NH_4 - N is released from the oxidisable organic matter in the form of ammonia gas. The liberated ammonia is collected in boric acid with mixed indicator and titrated against standard acid.

5. Available Phosphorus

Principle: Phosphorus from soil is extracted using an extracting solution (pH-8.5) .The extract containing available P is allowed to treat with acidic molybdate gives phosphomolybdate, which is on reduction with SnC12, develops characteristic blue colour. This intensity of blue colour depends upon the P concentration of the solution, which can be measured at 660 nm by spectrophotometer. The term available phosphorus incorporates both exchangeable and water soluble forms of the nutrient in soil. The readily exchangeable plus water-soluble potassium is determined in the neutral normal ammonium acetate extract of soil. Then, the estimation of the in the extract is carried out with the help of flame photometer.

6. Soil texture: The aim of textural analysis of soil is to determine the percentage of soil material contained in different size fractions and this can be done by means of mechanical analysis. Mechanical analysis consists essentially of two distinct operations, namely dispersion of the soil to ultimate soil particles and grading the dispersed particles according to their size groups.

Suggested Readings:

Soil and Water Quality Management in Brackishwater, C.I.B.A. special publication no. 13.

Clesceri, S. L ,Greenberg, E.A., Trussrl ,R.R., 1989. Standard Method For the examination of water and waste water, 17th edition

ISOLATION, IDENTIFICATION AND METHODS OF USE OF DIFFERENT BENEFICIAL MICROBES AS PROBIOTICS IN AQUAFEED

Debasis De, T.K.Ghoshal, Atanu Pramanik and Subha Ganguly

Like all vertebrates and invertebrates, fishes also harbor microbial populations in their digestive tracts. These populations grow upon the food absorbed by the host animal, digestive secretions and fragments scaled off the mucosal epithelium. The bacterial flora of the gastrointestinal tract in general, represents a very important and diversified enzymatic potential. These beneficial microbes can be incorporated in aquafeed as probiotics which will improve digestion and growth of fishes and also remove pathogens from their gut. The main strategy in the use of probiotics is to isolate intestinal bacteria with favorable properties from mature animals and include large numbers of these bacteria in the feed of immature animals of the same species.

1. Isolation of gut bacterial flora

Brackishwater fishes should be collected from natural habitat and should be starved for 48 hours to clear their alimentary tract (Fig.1). Immediately after being pithed and sacrificed, the ventral surface of the fish should be thoroughly scrubbed with 1% iodine solution. The fishes have to be dissected within laminar airflow (Fig.2) and their alimentary tracts have to be removed and cleaned with sterile chilled physiological saline (0.9% NaCl in PBS buffer, pH 7.2). Subsequently, the pieces of digestive tract (Fig.3) have to be homogenized with sterilized 0.9% NaCl solution (1:10; w/v). The homogenate is used as inoculum for microbial culture.

Fig 1

Fig.2

Fig 3

2. Microbial culture

The homogenate has to be diluted in different serials. Samples should be taken from each dilution and poured aseptically under laminar flow on selective media plates to screen beneficial microbes from total microbiota. Colonies isolated on the basis of their morphological appearances (Fig.4) should be streaked several times on the same medium to obtain pure culture and has to be maintained on selective medium slant at 4°C. For long time preservation, the pure culture of the isolates should be preserved using 15%

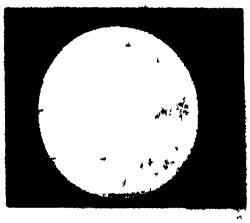

Fig.4

sterile glycerol. By multiplying the number of colonies formed on each plate by the reciprocal of dilution, total colony numbers per unit sample volume of gut homogenate is determined.

3. Morphological, physiological and biochemical characterization

Isolated microbes are characterized by studying the colony morphology, motility, growth characteristics at different temperatures, sodium chloride tolerance, catalase production, nitrate reduction, H_2S production, indole and urease production.

Morphological tests

Colony morphology of all the isolates formed on selective media plates have to be studied visually by hand magnifying lens for determining their configuration, margin, elevation, surface, density, texture and color. Cultural characteristics are observed in broth for turbidity, sedimentation and pellicle formation. Motility is studied by hanging drop method. Bacterial staining should be performed by using crystal violet, iodine solution, alcohol (abs.) and safranin (Fig.5). Size of all the bacterial isolates should be determined by stage and ocular micrometry. Capsular staining is done by using India ink and carbol fuchsin. Endospore formation by the isolates have to be studied by spore staining method with 5% aqueous malachite green and 0.5% aqueous safranin solution.

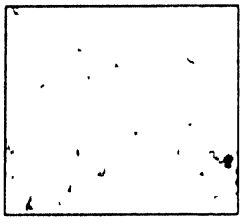

Fig.5

Physiological tests

Bacterial growth in Luria broth is observed for turbidity, sediment and pellicle formations. These are also observed under different culture conditions of varying temperature (5-60 °C) and pH (2.0-11.0). Salt tolerance (5-10% NaCl) should be determined by Davis and Mingioli medium for viability and growth.

Biochemical tests

Catalase producing capability is tested by pouring 3% aqueous solution of H_2O_2 drop-by-drop on 24 hours incubated broth culture with formation of effervescence on glass slide (Fig.6). Nitrate reduction is determined after 24 hours in the medium supplemented with KNO_3 (0.2% w/v), using α-napthylamine and sulphanilic acid in 5N acetic acid. The color of the broth changed to be red when the result is positive (Fig7). H_2S production is tested by inserting sterile lead acetate paper strips in nutrient broth medium. Indole production is determined by using Kovac's reagent. Positive test is shown by the formation of pink colored ring at the upper portion of the broth (Fig.8).

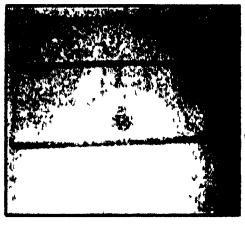

Fig.6

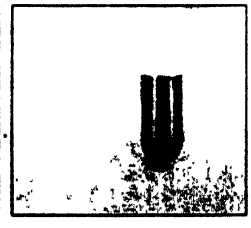

Fig.7

Citrate utilization is determined using Simmon's citrate medium containing citrate as sole carbon source. After positive utilization of citrate, the color of the medium changed to blue. For testing of urease, culture broth is inoculated with each isolates and after incubation; the urea hydrolysis is determined by phenol red which became violet red color due to hydrolysis of urea and increase in pH of the broth (Fig.9).

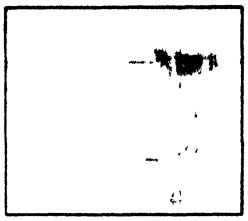

Fig 8

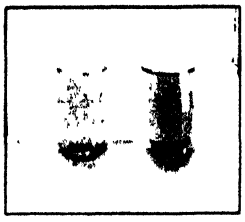

Fig.9

4. Methods of using beneficial microbes as probiotics in aquafeed

After assessing the digestive enzyme production potential and the probiont characteristics of isolates, microbes are screened for using as probiotics in aquafeed.

i) *In vitro* study to measure fibre and starch degradation of different aquafeed ingredients:

Different low cost fibrous feed ingredients e.g rice bran, wheat bran, ground nut cake, sunflower cake and other agro-industrial by-products are inoculated with each strain of isolated microbes and incubated for 24, 48, 72 and 96 h in triplicate. Fibre, fat and protein content of feed ingredients is measured before and after the incubation to estimate the *in vitro* degradation of fibre. For each ingredient, the experiment should be repeated thrice. Feed fermented by enzymatically potential microbes obtained from solid state fermentation (SSF) can also be used to study the growth performance of fishes in tanks.

ii) After screening of the enzymatically potential microbes they are tested for their virulent effect, if any, on brackishwater fishes.

iii) Only avirulent microbes are selected to study the viability of microbes as a feed supplement at different conditions of storage and at different hours.

iv) Viable beneficial microbes are selected to study the effect of microbial supplemented feed on growth performance of fishes. Different doses of microbial supplemented feed(s) are given to fishes (juveniles). Growth trial has to be carried out for selected period. Best dosage of microbial supplement should be selected based on the growth performance and feed digestibility of fishes in tanks.

Suggested Readings:

Jacob, M.B. and Gerstein, M.J. (1960). *Handbook of Microbiology*. Princeton, New Jersey: D Van Nostrand Co. Inc.

Pacarynuk, L. A. and Danyk, H. C. (2005) *Principles of Microbiology, Laboratory Manual*, Spring, The University of Lethbridge.

Williams, S.T., Sharp, M.E. and Holt, G.,(eds.) (1986) *Bergey's Manual of Systematic Bacteriology*, Vol. I., Baltimore, USA: Williams and Wilkins.

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PREPARATION OF CRAB FEED AND FEEDING STRATEGIES

T. K. Ghoshal and Debasis De

In India, mud crabs are extensively exploited from both the inshore marine and adjoining estuarine areas with specialized indigenous gears and are in the limelight due to their great demand for their delicacy, medicinal value and export trade. There are two species of mud crabs, namely, *Scylla tranquebarica* (larger species) and *Scylla serrata* (smaller species) which have high potentials for aquaculture. In India the mud crabs have come into prominence since early eighties with the commencement of live crab export to the South East Asian countries which has created a renewed interest in the exploitation as well as in the production of mud crabs through aquaculture. The importance of live mud crabs as an export commodity has opened up great opportunities for crab farming. It has high demand and price in the export market. The present exports are to a tune of Rs.46.2 crores. Mud crabs can tolerate wide range of salinities and migrate into estuarine areas during their post larval stages, grow fast and attain maturity. They are found in the lower, middle and upper reaches of estuarine system and also in the traditional fish/ shrimp culture fields of Kerala, Karnataka, Tamil Nadu and West Bengal. Among the marine crabs, mud crab is the only species which can remain alive out of water for considerable time. Culture of mud crabs in suitable enclosures is practiced for the last forty years in South-East Asian countries. Initially, juvenile mud crabs are cultivated along with milk fish (*Chanos chanos*) and later due to their great demand, monoculture practices are more prevalent. Presently the stocking materials are mainly drawn from wild but with the establishment of commercial hatcheries in Tamil Nadu, the culture is expected to flourish in a great extent in near future.

Feed and feeding habit of crabs

Mud crabs are omnivorous and they feed on a wide variety of food items such as shrimps, crabs, bivalve molluscs and fish etc. Feeding habit of crab is more or less same as shrimps and they are also slow feeder and mainly nocturnal in habit. Presently in most part of our country, crab culture is being practiced with trash fish or bivalve meat which is not a sustainable approach and uncertain due to high price fluctuation, seasonal variation and erratic supply of trash fish and the costs of these feeds continue to increase and in time its use may no longer be profitable. Formulated pelleted feed using cheap and indigenous feed ingredients is an alternative approach to popularize crab culture and CIBA has developed cost effective pellet feed for culture of mud crabs. Feed cost is considered the most expensive

single factor in the culture of mud crabs as it constitutes 40 to 50% [redacted] production. Feeding is done daily at the rate of 5 to 10% of body weight.

Nutritional requirements

Mud crabs also require all the nutrients like tiger shrimp. *P.monodon* and the major nutrient requirements are as follows: Crude protein: 35-40 %. Crude fat: 5-8 % and Crude fiber: 4-10%.

Feed ingredients used

Dry fish, acetes, prawn head waste, squid, squillq, soyabean cake, mustard [redacted], wheat flour, rice flour, maize flour, vitamins, minerals and binder.

Method of feed preparation

Grinding: First ingredients like dry fish, acetes, prawn head waste, squid waste, squilla [redacted] grinded in hammer mill to reduce the size. Then all the ingredients are powdered in a [redacted] pulverizer separately.

Mixing: All the ingredients are weighed as per the formula and put into the mixer except vitamin and mineral mixture. The feed mix is homogenised for 15 minutes. 35 litres of water are added to ithe mixture and further homogenized for another 10 minutes.

Steam cooking: Feed mixture is loaded in trays and the trays are kept in steaming chamber. Temperature is allowed to reach 95-100^{0}C and kept for 5 minutes. Then mixture is taken out and allowed to cool.

Incorporation of vitamins: The vitamin and mineral mixture is added to the steamed cooled feed mix and thoroughly homogenized in a dough mixer.

Pelletisation: The feed mixture is pelletised in a pelletizer fixed with 5 mm diameter die. The pellets are collected in aluminum trays.

Drying: The trays loaded with moist feed are kept into an electrical tray dryer. The temperature is adjusted at 75-80^{0}C and allowed the feed to dry until the moisture content is less than 10%.

Checking quality of feed: The dry feed pellets may be physically examined for general appearence such as uniformity, colour and smell. The pellets should have smooth surface without cracks. The feed may be sampled and analyzed for proximate composition.. The water stability of the pellets may also be tested after twenty four hours of preparation.

Composition of crab feed

Ingredients	Percentage
Fish meal	31.00
Acetes	7.00
Soyabean cake	15.00
Mustard cake	10.00
Wheat flour	20.00
Rice bran	8.00
Fish oil	3.00
Soyabean oil	1.00
Soya Lecithin	1.00
Mineral mixture	2.00
Vitamin mixture	1.00
Binder	1.00

Feed cost: Rs.20.00/kg and Feed Conversion Ratio (FCR):1.5-2 : 1

Feeding rate

Trash fish/ slaughter house offals (fresh basis) : 5-10% of total biomass

Pellet feed: 1.5-2.0% of total biomassd

Example: Feed requirement in 0.2 ha culture (@ 1 ps./sq m) pond when fed with trash fish–

Days of culture	No. of crabs stocked	Survival %	Average body wt. (g)	Feeding rate (%)	Total feed (Kg)
1-30 days	2000	100	90	5	270
31-60 days	2000	90	150	6	486
61-90 days	2000	80	225	8	864
91-120 days	2000	70	300	10	1260
				Total	2880

Suggested Reading:

Mud Crab Culture in India. CIBA Bulletin No. 17, 2004.

The Mud Crab-A report on the seminar convened in Surat Thani, Thailand, 1991 (BOBP/RE/51).

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ESTIMATION OF DIFFERENT DIGESTIVE ENZYMES OF SHRIMP AND FINFISH

Debasis De, T.K.Ghoshal, R. Ananda Raja, Atanu Pramanik and Subha Ganguly

Digestive enzyme assay

Both endogenous and microbial secretory enzymes constitute total digestive enzyme in the gut of shrimp and brackishwater fishes. After evisceration, the whole gut content is homogenized with five times (w/v) of ice cold sterile chilled physiological saline (0.9% NaCl in PBS buffer, pH 7.2). Homogenate is centrifuged at 10,000 x g for 1h at 4°C and the supernatant is collected and used for enzyme assay.

Amylase Assay

Amylase activity is measured using 1% soluble starch in phosphate buffer (0.02 M; pH 6.9 containing 0.0067 M NaCl) as substrate. 1 ml of crude enzyme is incubated for 5 mins at 37^0C with 1 ml of substrate solution. The enzyme reaction should be interrupted by the addition of 1 ml of dinitrosalisylic acid (DNS) reagent. The tube containing this mixture should be heated for 5 mins in boiling water bath and then cooled in running tap water. The production of reducing sugar (maltose) from starch substrate because of amylolytic activity should be measured at 540 nm by dinitrosalisylic acid method using maltose as the standard. The blank should be prepared in the same manner using 1ml sterile distilled water in the place of the crude enzyme. One amylase unit is defined as the amount of enzyme per milliliter culture filtrate that released 1 μg maltose per minute.

Cellulase Assay

Cellulase activity is assayed using 1% CMC in citrate buffer (0.1 M, pH 6.75) as substrate. 0.5ml of crude enzyme is incubated for 15 mins at 37^0C with 1 ml of substrate solution. The enzyme reaction should be interrupted by the addition of 0.5 ml of dinitrosalisylic acid (DNS) reagent. The tube containing this mixture is heated for 5 mins in boiling water bath and then cooled in running tap water. The production of D-glucose from cellulose because of cellulolytic activity should be measured at 540 nm by dinitrosalisylic acid method using D-glucose as the standard.The blank is prepared in the same manner using 0.5ml strerile distilled water in the place of the crude enzyme. One cellulase unit is defined as the amount of enzyme per milliliter culture filtrate that released 1 μg D-glucose per minute.

Protease assay

Protease activity is detected by caseinase assay method. Protease activity is assayed using 0.5% casein in Tris HCl buffer (0.02 M, pH 7.0) as substrate. 0.5ml of crude enzyme is

incubated for 20 mins at 37^0C with 1 ml of substrate solution. The enzyme reaction should be interrupted by the addition of 1.5 ml of tricarboxylic acid (TCA) soln. The tube containing this mixture is centrifuged at 10,000 g for 5 mins. After centrifugation, 0.5 ml supernatant is taken and subjected for protein content estimation using L-tyrosine as standard. The blank should be prepared in the same manner using 0.5 ml sterile distilled water in the place of the crude enzyme. One unit of enzyme activity represents the amount of enzyme required to liberate one microgram of tyrosine per milliliter culture filtrate under standard assay conditions.

Protein content estimation

Estimation of protein content is done using 0.2 mg/ ml Bovine serum albumen (BSA) as standard protein solution, alkaline reagent (mixture of alk. sod. carbonate with copper sulfate and sod. pot. tartrate soln in the ratio of 50:1), and Folin-Ciocalteu reagent (1:1 aqueous soln.) as a coloring agent spectrophotometrically. 0.5ml alkaline reagent is mixed with 0.1 ml protein soln. and allowed at room temp. for 10 mins. After that 0.05 ml of Folin-Ciocalteu reagent should be mixed with that mixture and allowed at room temp. for 30 mins After that free L-tyrosine released in the reaction mixture is measured at 750nm against blank. The blank should be prepared using 0.1 ml sterile distilled water instead of standard protein solution.

$$\text{Specific enzyme activity (Unit of activity/ mg of protein)} = \frac{\text{Enzyme activity}}{\text{Protein content of that enzyme}}$$

Suggested Readings:

Bernfield, P. (1955) Amylase [alpha] and [beta] In *Methods of Enzymology*, Vol. I. Colowick, S.P. and Kaplan, N.O (eds). New York, USA: Academic Press, pp.149.

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IDENTIFICATION OF MAJOR PHYTOPLANKTON GROUPS PRESENT IN BRACKISHWATER PONDS

P.S. Shyne Anand, Sujeet Kumar and A. Panigrahi

Introduction

Planktons are drifting and floating aquatic organisms with limited power of locomotion and transported primarily by water currents. Planktons are mainly of two types. phytoplankton and zooplankton. The phytoplanktons are mainly unicellular plants known as algae. They are found dispersed throughout the photic zone of the aquatic ecosystems and account for the major share of primary productivity in the brackish water ponds. The major phytoplankton group includes diatoms, dinoflagellates, blue green algae and green algae. Plankton is classified according to their size. These size groups are ultra plankton(less than 2 µm) nanno plankton (2-20 µm), microplankton (20-200 µm), and magaplankton (more than 2000µm).

A. Class Bacillariophyceae (Diatoms)

- Occur as single cells or in chains or other loose aggregates
- cell wall (frustule) made mostly of silica and consisting of two closely fitting halves (epitheca and hypotheca)
- Major photosynthetic pigments are Chlorophyll a, Chlorophyll c and Fucoxanthin. The major storage product is fat globules.
- Circular, triangular, with radially symmetric are known as centric diatoms. Pennate diatoms have a bilateral symmetry in cell form.
- Centric diatoms: *Coscinodiscus*, *Chaetoceros* (chain), *Thalassiosira* (chain), *Planktoniella*, *Triceratium* and*Cyclotella*. Some common pennate diatoms are *Pleurosigma*, *Gyrosigma*, *Rhizosolenia*, *Thalassiothrix* (chain), *Thalassionema* (chain), *Diatoma*, *Navicula*, and *Nitzchia*.

B. Class Dinophyceae (Dinoflagellates)

- Presence of a porous cell wall made mostly of cellulose. In some armored forms, the cell wall consists of many articulating cellulose plates arranged irregularly over the cell surface most possess several small chloroplasts usually located near the cell ends.
- Typical forms have a body surface with two grooves; each having a flagellum may possess spines, horns or other projections.
- Main photosynthetic pigments are Chlorophyll a, Chlorophyll c and Peridinin. Storage product is made of starch.

Representatives: *Gymnodinium, Peridinium, Ceratium, Dinophysis,* and *Gonyaulax*

C. Class Cyanophyceae (Blue green algae)

- Blue green algae ***or Cyanobacteria*** are single cells, colonial forms or filaments of cells (trichomes).
- Poorly organised or diffused nucleus with prokaryotic cell organisation
- Photosynthetic pigment is Chlorophyll a, billproteins, phycocyanin
- Heterocyst assists in nitrogen fixation.

Representatives: *Microcystis, Ocillatoria, Lyngbya, Anabaena, spirulina*

D. Chlorophyceae (Green algae)

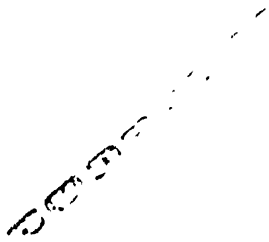
- Unicellular or multicelluar filamentous ,branched or unbranched
- Photosynthetic pigment is Chlorophyll a and b
- Storage form is starch and cellulosic cell walls.

Representatives : *Volvox, Enteromorpha, Chlorella, Cosmarium, Chaetomorpha, Ulothrix*

Brackish water Phytoplankton Identification Key

A. Class: Bacillariophyceae

1. *Skeletonema costatum*



Order: Biddulphiales
Family: Thalassiosiraceae

- Cells cylindrical with rounded ends
- Cells form long straight chains, held together by fine marginal processus.
- Spines are straight and slender and unite with the spines of the next cell to form a junction.

2. *Thalassiosira* sp.

Order: Biddulphiales
Family: Thalassiosiraceae

- Girdle view of cells is rectangular, valve face round and flat.
- Cells connected by connecting threads.

3. *Melosira* sp.

Order: Biddulphiales
Family: Melosiraceae

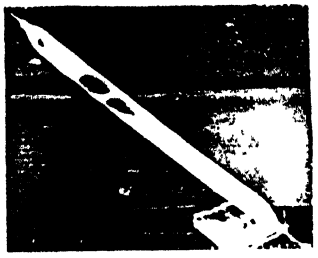
- Cells are connected to form long chains, looking like a string of beads.
- Several plate-like chromatophores. Nucleus is central.

4. *Coscinnodiscus* sp.

Order: Biddulphiales
Family: Coscinodiscaceae

- Cell is asymmetric, one side much higher than the other.
- Solitary cells, valves are arched or flat
- Fine lines radiating from a definite central rosette

5. *Rhizosolenia* sp.



Order: Biddulphiales
Family: Rhizosoleniaceae

- Cells cylindrical. Valves oblique and pointed.
- Apical process hollow nearly all the way, with small wings at the base which run up to about a third of the spine.

6. *Chaetoceros* sp.

Order Bidulphiales
Family Chaetoceroceae

- Long thin setae originate at corners, united to form 4-8 cells per chain.
- Cell surface concave, Setae originate at cell corners but are directed in different directions.

7. *Navicula* sp.

Order. Bacillariales
Family: Naviculaceae

- Elongated cells
- Pointed at both ends, two plate-like chloroplasts.

8. *Pleurosigma* sp.

Order: Bacillariales
Family: Naviculaceae

- Cells elongated and sigmoid.
- Raphe more or less sigmoid and central

9. *Nitzschia* sp.

Order: Bacillariales
Family: Bacillariaceae

- Cells solitary and slightly bend.
- Ends of bent horns are hair-like.

B. Class Dinophyceae

10. *Dinophysis* sp.

Order Dinophysiales
Family: Dinophysiaceae

- Cell oval or elliptical in shape.
- Left sulcal list is well developed, extends beyond the midpoint of the cell.
- Posterior profile of hypotheca is rounded. Epitheca is dorsoventrally reduced

11. *Gyrodinium* sp.

Order. Gymnodiniales
Family: Gymnodiniaceae

- Large spindle shaped asymmetric cell with slight longitudinal twist.

12. *Ceratium* sp.

Order: Gonyaulacales
Family: Ceratiaceae

- Pentagonal cell shape.
- Epitheca forming a more or less equilateral triangle with long apical horn.
- Hypotheca extends into two unequal horns pointing in opposite directions

13. *Gonyaulax* sp.

Order: Gonyaulacales
Family: Gonyaulacaceae

- Cells thecate, elongated and tetragonal in dorso-ventral view.
- Hypotheca bearing two spines.
- Epitheca with convex sides leading into an apical horn

C. Class : Cyanophyceae

14. *Microcystis* sp.

Order: Chroococcales
Family: Chroocaecae

- Round colony with thick gelatinized mucilage. 50-70µm broad.
- Single cell is Spherical or ellipsoid and 3-5µ m

15. *Lyngbya* sp.

Order: Nostocales
Family: Oscillatoriaceae

- Filamentous, trichome with prominent sheath.
- Free thallus, presence of cross walls in the cells

16. *Oscillatoria* sp.

Order: Nostocales
Family: Oscillatoriaceae

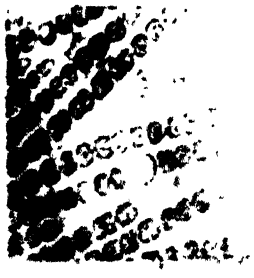
- Trichome without sheath and more or less straight.
- Filamentous forms, non restricted cross walls.

17. *Spirulina* sp.

Order : Nostocales
Family:Oscillatoriaceae

- Unicellular or multicellular. Spirally coiled, sheath absent. terminal cells rounded

18. *Anabaena* sp.

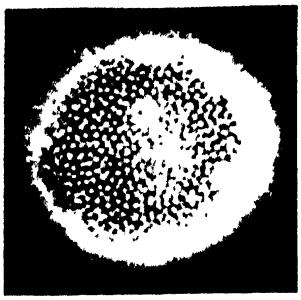


Order : Nostocales
Family:Nostocaceaae

- Filamentous, single or gelatinous mass, heterocyst present,single cell barrel shape.

D. Class: Chlorophyceae

19. *Volvox* sp.



Order: Volvoccales
Family: Volovocaceae

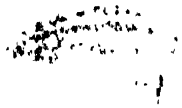
- Collonial thallus. cells held together in gelatinus matrix. single cells spherical. motile cells

20. *Enteromorpha* sp.

Order: Volvoccales
Family: Ulvaceae

- Tubular. elongated thallus. branched. attached to holdplast.

21. *Closterium* sp.



Order: Zygnematales
Family: Desmidiaceae

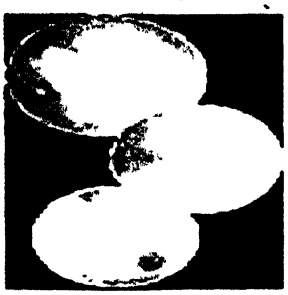
- Cells solitary elongate. without median construction . attenuated at the poles

22. *Cosmarium* sp.

Order: Zygnematales
Family: Desmidiaceae

- Unicellular with compressed cells. length ids equal or greater than breadth. with median construction

23 .*Chlorella* sp.



Order: Chlorococcales
Family: Oocystaceae

- Cells are solitary, spherical, with smooth cell wall, single chloroplast

24. *Chaetomorpha* sp.

Order: Cladophorales
Family: Cladophoraceae

- Filamentous unbrached or with short branch lets, attached by basal ends.

Suggested Reading:

Neera Sen and Kumudranjan Naskar (2003) Algal flora of sundarbans mangal. Daya publishing House, 317 pp.

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FEED PREPARATION FOR SHRIMP AND FINFISH

T.K.Ghoshal and Debasis De

Shrimp farming has shown phenomenal growth in the last decade in India producing protein rich health food and earning valuable foreign exchange. Feed is a major input in shrimp farming. Preparation of nutritionally adequate feed for tiger shrimp (*Penaeus monodon*) involves understanding the dietary requirements of the species, proper selection of feed ingredients, formulation of feeds and appropriate processing technology for producing water stable pellet feeds. Depending upon the type of farming, a wide range of feedstuffs are used for feeding stocked shrimp. While no feed is used in traditional farming systems, supplementary and adequate feeds are used in improved extensive aquaculture. To formulate a practical diet for tiger shrimp and fish, first and foremost point to be considered is the nutrient requirement of the species. Shrimp diet should have adequate energy and protein to meet the requirement for maintenance and growth. In nature, shrimp can meet their requirement from a variety of feed available in the ecosystem. But when shrimps are cultured in confined systems, they should be provided with balanced diet as close to natural feed as possible.

Different ingredients used for shrimp and fish feed

Wheat flour, rice flour, maize flour, soybean cake, ground nut cake, cotton seed cake, sun flower cake, fish meal, prawn meal, prawn head meal, squilla, squid, clam meal, cuttle fish, meat meal, silk worm pupae meal, shark liver oil, cod liver oil, fish oil, soybean oil, soyalecithin, sunflower oil, safflower oil, brewer's yeast, spirulina, mineral mixture, vitamin supplement and binder (guar gum, cellulose, hemicellulose and synthetic binder) are the ingredients generally available for selection and use for formulation of shrimp and fish feed.

Table 1. Different ingredients of plant origin with nutritional value

Ingredients	As % dry matter			
	Crude Protein	Crude Lipid	Crude Fibre	Total Ash
Soybean cake	42-48	2-7	6-8	5-7
Ground nut cake	40-43	3-8	6-9	4-8
Cotton seed cake	36-44	4-8	16-22	6-9
Sun flower oil cake	38-47	4-6	14-16	6-7
Wheat flour	9-12	3-4	6-8	4-6
Maize	10-12	4-6	4-8	6-9
Tapioca	1.5-2.5	0.4-0.6	2-6	2-4
Brewer's yeast	40-45	1	2-7	6-9
Spirulina	55-68	6-8	1-3	8-10

Table 2. Different ingredients of animal origin with nutritional value

Ingredients	As % dry matter			
	Crude Protein	Crude Lipid	Crude Fibre	Total ash
Fish meal	52-60	5-12	1-4	22-38
Prawn meal	58-65	4-7	4-7	21-26
Prawn head meal	34-45	4-7	11-18	36-44
Squilla	37-40	4	-	23
Clam meal	40-58	6-12	-	5-9
Cuttle fish	67	5	1	12
Meat meal	44-52	8-11	2-4	24-31
Silk worm pupae meal	48-53	26-30	6-8	7-11

Feed preparation procedure

To prepare a batch of shrimp feed using small scale feed mill equipment, the following procedure may be followed

Grinding: Individual ingredients e.g. dry fish, prawn head waste, squid waste; soybean meal etc. should be ground in a hammer mill (Fig.1) to reduce the particle size. Then all the ingredients should be powdered in a pulverizer (atta chakki) (Fig.2) and then in micro pulverizer (Fig.3) separately.

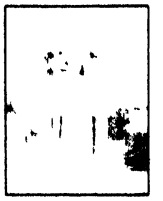

Fig 1 Hammer mill

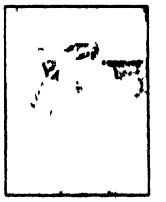

Fig 2 Pulverizer

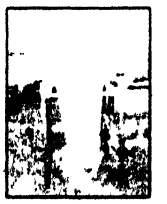

Fig 3 Micropulverizer

Mixing: As per the formula, feed ingredients to be weighed and mixed mechanically with the help of a horizontal mixer (Fig. 4) or manually by hand except vitamin and mineral mixture. Sufficient quantity (35-45 litres /100 Kg feed) of water should be added and mixed thoroughly.

Steam cooking: Feed mix has to be kept in steaming chamber. If steaming chamber is not available, the mixture is to be cooked in a big container at 100 °C temperature for 5-10 minutes.

Incorporation of Vitamin-Mineral mixture: Vitamin and mineral mixture to be added in the steamed and cooled feed mix and thoroughly homogenize in a dough mixer or manually by hand (Fig. 5).

Fig.4 Horizontal blender

Fig.5 Manual mixing of micronutrient

Pelletisation: The feed mixture has to be pelletised in a pelletizer (Fig. 6) using desired die size.

Drying: Moist pellet is to be collected in the aluminum trays and kept into an electrical dryer (Fig.7) at 60-70 ^{o} C temperature and allow the feed to dry until the moisture content is less than 10%. Where the dryer facility is not available, pellet may be dried under the sunlight. After drying pellet feed has to be crumbled with crumbler (Fig. 8) to get desired sizes of pellet.

Fig 6 Pelletizer

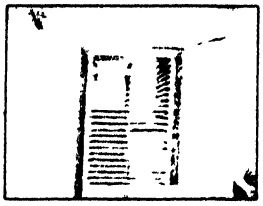

Fig 7 Dryer

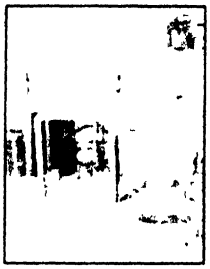

Fig 8 Crumbler

Checking quality of feed: Dried pellet feed should be physically examined for visual appearance such as uniformity, color and smell. The pellet should have surface without cracks. Feed may be sampled and analyzed for proximate composition. Water stability of the pellet may also be tested after 24 h of preparation.

Storing: Dried pellet feed should be packed properly in polythene bags and kept on raised wooden platform to avoid absorption of moisture.

Central Institute of Brackishwater Aquaculture (CIBA), Chennai and it's regional centre at Kakdwip extend technical guidance to set up feed mills in West Bengal and Tamilnadu for preparation of shrimp feed using ingredients available in the country

Suggested Reading:

Hardy. R.W. and F.T. Barrows. (2002) Diet formulation and manufacture, In: fish nutrition by J.E. Halver and R W.Hardy. 3rd Edn. Academic Press.

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