TRAINING MANUAL
SEPTEMBER 2021

CIBA-TM-Series-2021-
No.18, 2nd Edition

HANDS-ON TRAINING ON SHRIMP AND MUD CRAB CULTURE:
A Practical Exposure

ICAR-Central Institute of Brackishwater Aquaculture
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(ISO 9001:2015 certified)

Training manual
Hands-on Training on Shrimp and Mud crab Culture: A Practical Exposure

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Hands-on Training on Shrimp and Mud crab Culture: A Practical Exposure

Brackishwater aquaculture for food, employment and prosperity

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Cite as: Balasubramanian C. P, Jayanthi M, A. Panigrahi & et al., Hands-on Training on Shrimp and Mud crab Culture: A Practical Exposure, Training manual series, no-18, *2nd Edition*, 2021, 176 pp.

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Grow-out production systems for shrimp and mud crab

Biology of brackishwater candidate species with special reference to *Penaeus vannamei*

Balasubramanian, C.P., Biju I.F., Shyne Anand, P.S and Sudheer N.S

Aquaculture is the farming of fish, crustaceans, molluscs and aquatic plants in the aquatic environment; sometimes, it is referred to as aquatic agriculture, as an aquatic counterpart of terrestrial agriculture. Here farming implies some sort of intervention in the rearing process, such as regular stocking, feeding or protection from predators. Aquaculture is the fastest growing food producing industry with a total global aquaculture production of 73. 8 million tonnes. A total of 582 species are farmed worldwide, and of these, 62 are crustaceans. The total global farmed crustaceans was 6.9 million tonnes valued at 37 billion USD. Although many crustaceans attract lucrative markets, shrimp has become the single most successful crop and mainstay of the brackishwater coastal aquaculture in India and many Asian countries. Aquaculture of shrimp is considered to be a success story of modern aquaculture. Shrimps had been raised as an incidental crop in coastal ponds/or coastal low lying ecosystems, including India. The advent of sophisticated refrigeration facilities provided by artisanal farmers accessed by international markets. Thus traditional coastal aquaculture shifted to an export oriented or industrialized aquaculture. Farmed shrimp production has shown remarkable growth during the last 25 years, from almost 50000 mt in 1990 to 600000 mt in 2016. Tiger shrimp, *Penaeus monodon*, and Pacific white shrimp *Penaeus vannamei* are the most important farmed shrimp across the world. Although *P. monodon* was dominating species, since 2001, global shrimp aquaculture dramatically shifted to *P. vannamei*, because of the availability of disease free stock. It is paramount to have a basic and solid knowledge of the biology of the species to be cultivated in order manage the production system efficiently and optimizing the profitability of the farming. The present lecture note is intended to provide basic biological knowledge with regard to shrimp farming.

Nomenclature and Taxonomy

Shrimp versus prawns: These two words are used synonymously in many literatures, despite the consensus arrived at the world conference on biology and culture of shrimps and prawns held in Mexico City in 1967 to restrict the term 'prawn' to freshwater forms and 'shrimps' to marine and brackishwater counterpart. There are no technical differences between shrimp and prawns. However, presently in the Indian context, we restrict to the consensus of FAO and use the word shrimp for all the marine and brackishwater species. Three general groups of shrimps and prawns are found: Penaeid, caridean and stenopodean shrimp.

Penaeids: Almost all aqua cultured shrimps are penaeid shrimps (of the genus *Penaeus*). The first three pereopods or walking legs are chelate and of similar size and shape. The pleuron of the second abdominal segment overlaps with the third but not with the first. Females of this group release eggs directly into the water.

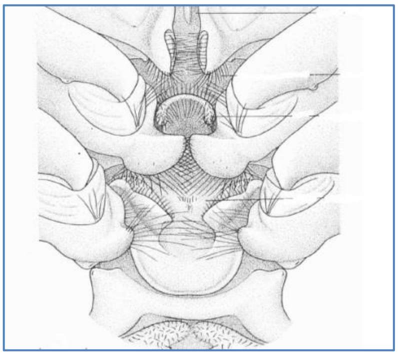
Caridieans: The third pair of this group is not chelate; the pleura of the second abdomen overlaps the first and third abdominal segment. Female carries the eggs until hatching

Stenopodean shrimp: Lesser known shrimp, third pereopod is chelate and considerably long, female carry eggs and pleara of the second abdomen is similar to penaeid shrimps

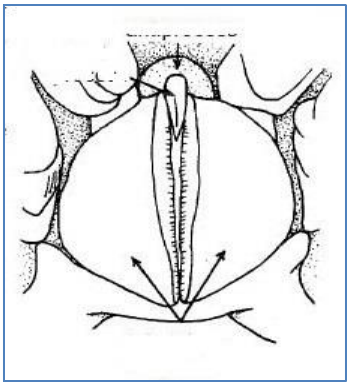
Created in 1798, the genus *Penaeus* has 29 species. Shrimps of this genus always received attention as they are commercially cultured and economically relevant to many countries. Until 1997, these 29 species were included in the same genus, *Penaeus*, and in 1997 Perez Farfante and Kenesely reclassified this genus into six independent genera: *Litopenaeus, Farfantepenaeus, Fenneropenaeus, Penaeus, Melicertus, and Marsupenaeus*. All the open thelycum (female external reproductive organ) species are included under the genus *Litopenaeus*. This morphological difference in the reproductive morphology and associated reproductive behaviour was considered as one of the major arguments for the reclassification of the genus *Penaeus*. However, the subsequent molecular studies carried out by researchers worldwide, including researchers from CIBA, proved that there is no sufficient evidence for splitting the genus *Penaeus*. Currently, all 29 species are included in the genus *Penaeus*.

Biology

Commercially important farmed shrimps of genus Penaeus can be grouped into two groups based on the reproductive morphology of female shrimp. Females of all shrimps and prawns have a specialized region on the ventral side between the third and fifth pereopods/ walking legs. It serves as a genital area for genital contact of males where they deposit the spermatophores or sperm sac during mating, and the female carries these spermatophores either temporarily or for a prolonged duration. This region is termed as thelycum, the Greek meaning is 'women like', and it is unique in each species and sometimes used as a diagnostic character for species identification. In all Asian species of genus *Penaeus* this thelycum is highly specialized closed structure, closed thelycum species (for example *P. monodon, P. indicus, P. merguiensis, P. penicillatus ,P. japonicus, P. semisulcatus* etc) whereas five species inhabiting in Pacific and Atlantic species (*Penaeus vannamei, P. stylirostris, P. occidentalis, P. schimitii and P. setiferus*).



Open thelycum



Closed thelycum

This structure comprises only ridges or hairs to keep spermatophores for a temporary period, and these species are denoted as open thelycum species.

Penaeus monodon: This species is widely distributed in Indo-west Pacific Ocean (from Japan to Australia), It was the species dominating in the shrimp aquaculture industry until

2000, and it was about 90% of total farmed shrimp production. It is the largest (363 mm maximum length) and fastest growing (up to 5. 5 g per week) shrimp. General body colour reddish brown (grey In bracklshwaters) with whitish and dark reddish brown (black in bracklshwaters) transverse. Carapace with 3 whitish transverse bands on the dorsal side. Antenna1 flagella is uniformly reddish brown without bands. Swimming legs fringed with bright red setae (dull red in bracklshwaters) and have white patches (yellow and blue In brackishwater) on the basal segment. Adults are often found on muddy sand or sandy bottom at 20 to 50 m depth in offshore waters. Wild males possess spermatozoa at 35 g size onwards whereas female becomes gravid from 70 g onwards. These animals are highly fecund, and lay 0.5 million to 7.5 million eggs. Although larvae are planktonic, juveniles and adults feed on detritus, polycaheate and crustaceans. The breeding and culture technology of this species was standardized by Tungkang Marine Laboratory in Taiwan and partly by Aquacop (Aquaculture team of Centre Océanologique du Pacifique) during 1972 1974. The intensive culture of this species was boomed from 1980 to 1988 in Taiwan due to the success in the development of formulated feed. However, the industry is collapsed in Taiwan due to unknown viral diseases). Subsequently, Thailand replaced Taiwan and became the top producer of *P. monodon*, and tiger shrimp culture spread to most south east Asian countries. This species became much sought after farmed shrimp during the 90s. However, frequent outbreaks of disease and crop failures forced shrimp farmers to seek alternatives. Drugs or chemicals cannot treat the disease, and exclusion of pathogens from the system is the only alternative to prevent disease. As monodon farming has been exclusively depends on the post larvae produced by wild broodstock, exclusion of pathogen is almost impossible. Domestication and production of spf P. monodon has still been an enigma for *P. monodon*. Thus the shrimp farmers of south east Asian countries depend the exotic SPF pacific white shrimp, *P. vannamei*. This shift in the farming revolutionized the shrimp industry, and *P. vannamei* became the top most farmed shrimp.

Indian white shrimp, *Penaeus indicus*: This species is native to the Indian Ocean from southern Africa to northern Australia and to all of south-east Asia. Body i$ light cream to yellowish with minute dots of red, yellow or black colour more or less evenly spread over the body. No bands on carapace and abdomen. Setae of swimming legs reddish. Antennal flagella reddish brown in the proximal half but yellow or white in the distal half. Rostrum is long and sigmoid in shape. The maximum size recorded in the wild is 100 g for females and 55 g for males; in the semi intensive culture, it will reach about 20 to 24 g within 100 days.

Banana shrimp, *P. merguiensis*: It is widely distributed in the Indo-West Pacific region in both tropical and subtropical waters. The body is similar to Indian white shrimp, *P. indicus*, which can be differentiated from *P. indicus* by a straight rostrum with a broadly triangular prominent crest at the base. The maximum size recorded for females is 120 g, and males are 60 g. In salt pans of Kutch it grew up to 33 to 37 g.

Penaeus japonicus: This species is distributed from the red sea, east and south Africa to Korea and Japan, southwards to Indonesia, North and North East Australia and Eastwards to Fiji. It is also present on the south east coast of the Mediterranian sea. This species is considered to be the most high valued shrimp because of the high demand in the Japanese market.

Penaeus vannamei

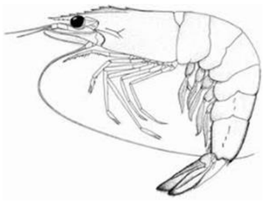
Taxonomic classification of *Penaeus vannamei* is as follows:

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Crustacea
Class	Malacostraca
Subclass	Eumalacostraca
Superorder	Eucarida
Order	Decapoda
Suborder	Dendrobranchiata
Superfamily	Penaeoidea
Family	Penaeidae
Genus	*Penaeus*
Species	*Penaeus vannamei* (Boone, 1931)
English name	Whiteleg shrimp

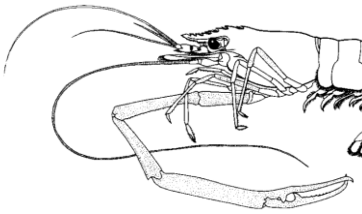
"Years before farmers discovered *Penaeus vannamei*, a zoologist named Willard Gibbs Vanname had collected the first specimen. The Yale professor was best known for his definitive monograph on sea squirts, his work with terrestrial and freshwater isopods and his work in ornithology. In the obscure world of museum curators and carcinologists (those who study shrimp, crabs and lobsters), history records that on March 25, 1926, Dr. Vanname purchased a male white shrimp in the fish markets of Panama City, Panama, and pickled it for the American Museum of Natural History collection, where he was curator of marine invertebrates. There it sat for five years, having turned red in the jar of alcohol, until a staff biologist at the museum, Miss Pearl Lee Boone, described it as a new species. Apparently, she admired Dr. Vanname, so she named it vannamei after him. She declared it to be the analogue of the North American white shrimp, *Litopenaeus (= Penaeus) setiferus*, that Linnaeus had described two centuries earlier. Her paper went on to detail the spine and eyestalks and measured its legs, pinchers and male sexual organs."

Years before farmers discovered the potential of farming of vannamei; the species was described in zoological literature. Professor Willard Gibbs Vanname, first obtained this species from a fish market in Panama City on March 25, 1926. Subsequently, this species was described by Miss Pearl Lee Boone, and named this species after Dr Vanname.

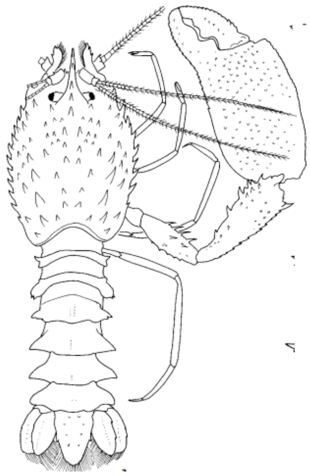
Description: Coloration is normally translucent white but can change depending on substratum, feed and water turbidity. Rostrum moderately long with 7-10 dorsal and 2-4 ventral teeth. In mature males petasma is symmetrical and semi-open. Spermatophores are



Penaeid shrimp



Caridean shrimp



Stenopodean shrimp

Figure Three groups of shrimps: Penaeid, caridean and stenopodean shrimps

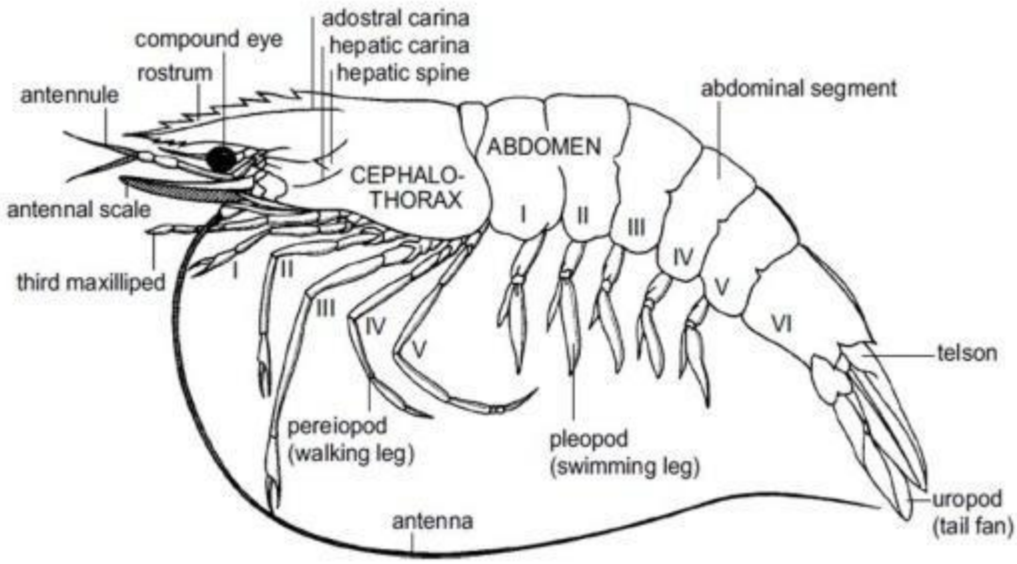


Figure Penaeus vannamei: External morphology

complex, consisting of sperm mass encapsulated by a sheath. The mature female has open thelycum. Maximum size 23 cm, with maximum CL of 9 cm. Females are commonly faster growing and larger than males.

The shrimp is native to the Eastern Pacific coast from Sonora, Mexico in the North, through Central and South America as far south as Tumbes in Peru. They are highly euryhaline and can withstand salinities ranging from 0 to 55 ppt. Adults live and spawn in the open ocean, whereas post larvae migrate inshore to spend their juvenile, adolescent and sub-adult stages in coastal estuaries, lagoons or mangrove areas. Males reach a total length of 187 mm and become sexually mature from 20 g onwards. Females are bigger with a length of 23 mm and reach sexual maturity from 28 g onwards at the age of 6-7 months.

External morphology of *P.vannamei*

The morphology of vannamei is the typical of a penaeid shrimp. The body of the animal is divided into 19 segments. The first 5 pairs are found in the cephalic region, the next 8 pairs are in the thoracic region, and last 6 pairs are found in the abdomen region. The head (cephalic) and thoracic region are joined together and form cephalothorax. Last portion after the abdomen is called telson, and it is surrounded by two pairs of uropods. Telson and uropod are together called tail fan

The life cycle of *P. vannamei*.

This species is distributed between Peru and Mexico; it prefers tropical marine habitats with a temperature above 20° C. *Penaeus vannamei* shows a similar pattern of life cycle of a typical penaeid shrimp. The adults live in the sea and reproductively mature in this environment. Females of *P. vannamei* grow faster than males; an adult female of the size of 35 to 45 g spawns 100000 to 250000 eggs. They lay eggs in the sea, and larval development takes place in this habitat. Post larvae drift towards the coast and enter into the less saline (brackishwater environment) ecosystem such as estuaries, brackishwater creeks and lakes. They grow into juveniles, and after spending 4-6 months in this environment, sub adults migrate back to the sea where they grow and attain sexual maturity and complete life cycle. The natural ability of the post larvae and juveniles to live and grow in the fluctuating salinity conditions of the estuarine environment has been made use of culture them in brackishwater ponds

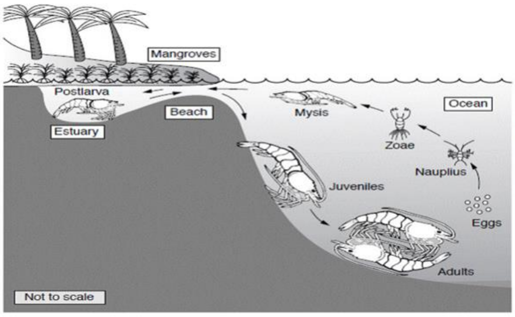


Figure 4. Penaeid life cycle (source: Rosenberry, 2009)

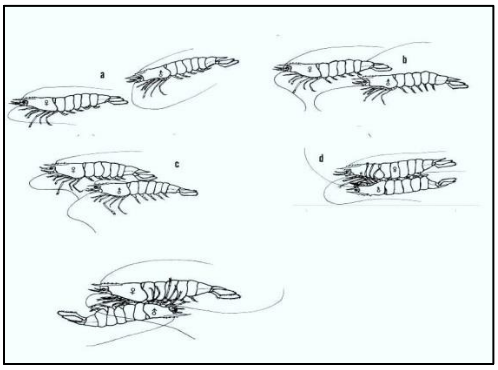


Figure: Mating in *P. vannamei*

Reproduction

Sexes are separate and are easily distinguishable through the external genitalia: the thelycum in females and petasma in males. Males and females can externally be distinguished even at a very early stage. Male possess a small rudimentary petasma, and thelycum of the female is discernible as a small elevation between fourth and fifth pereiopods. The males are generally mature at a smaller size than females. The male genital system comprises paired testes, paired vas deference and paired terminal ampoule. In addition to these internal organs, a paired petasma and paired appendix musculina are found externally. The female reproductive system consists of paired ovaries and paired oviduct. The ovaries are partly fused, bilaterally symmetrical bodies extending almost entire length of females in mature females. The seminal receptacle of the female is called the thelycum and consists of modified sternal plates on the seventh and eighth thoracic segments. The structure of the thelycum is unique to each shrimp species and is widely used in taxonomy. In white shrimp, like *P. vannamei*, thelyca are simple open depressions referred to as “open” thelyca.

In males, the development of gametes (spermatozoa or sperms) is relatively straightforward without the intervention of the growth phase (vitellogenesis: accumulation of egg yolk protein). While in oviparous females (egg lying), the gametogenic cycle is interfered by the growth phase, vitellogenesis. The process and control of vitellogenesis is very complex involves several physiological and nutritional pathways. Because of the accumulation of yolk protein into the oocytes, the ovary grows in size and changes colour. This characteristic feature of vitellogenesis enables to classify of the ovary into five maturation stages.

Hormonal control of reproduction

Endocrinological/neuro-endocrinological studies on crustaceans started in the first half of the 20th century. Generally, in invertebrates, oogenesis can be stimulated by a release from inhibition or by secretion of a stimulator. Ovarian maturation (oogenesis) in Crustacea is said to be stimulated by the gonad stimulating hormones secreted by the brain and thoracic ganglia and inhibited by Gonad Inhibiting hormones (GIH) of the eyestalk. The antagonism of eyestalk may be reduced by a decline in the titre of the GIH as the shrimp grows and moves into an environment suitable for spawning. The final spawning act may, in fact, be triggered by a stimulus, either visual or hormonal, originating in the eyestalk. Panouse (1943) demonstrated that the removal of the eyestalk of palaemonid shrimp would lead to ovarian development and spawning. However, the first use of the eyestalk ablation procedure for the inducement of reproductive maturation is successfully conducted by the Aquaculture team of Centre Océanologique du Pacifique (Aquacop). Alain Michael, the head of the Aquacop, serendipitously found that marine shrimp, *Penaeus aztecus*, who had lost the eye, matured and spawned in the tank. This observation he connected with the work of Panouse, and developed the most revolutionized technology in the captive breeding of shrimp. It had far reaching impact on crustacean aquaculture in general and penaeid shrimp farming in particular. The first successful maturation and spawning of P. monodon was achieved by Santiago (1977), although Alikunhi et al. (1975) achieved maturation with unviable spawning. The great majority of the captive maturation has been from ablated females, although few workers have reported maturation in unablated females (Santiago, 1977, Primavera, 1978. Emmerson, 1983), only Emmerson (1983) was successful in obtaining viable spawning (16.7 to 82% hatch rate). Although eyestalk ablation started as a stop-gap procedure to induce maturation and spawning in penaeid shrimp in the early 1970s, this procedure has been continuing in the commercial seed production industry across the world.

The most acknowledged consensus of crustacean reproductive endocrinology is two antagonistic hormones control that reproduction, one inhibits (Vitellogenin inhibiting hormone or Gonad inhibiting hormone, V/GIH) and other stimulates (Vitellogenin stimulating hormone, Gonad stimulating hormone, V/GSH). This simple endocrine axis has been questioned by many recent researchers and postulated a multi hormonal system involving several neuroendocrine and endocrine pathways, involving neurotransmitters (serotonin or 5 hydroxytreptamine), steroids (progesterone, estradiol), terpenoids(methyl farnesoate) and vertebrate peptide hormones (GnRH). Additionally, the role of other neurohormones in the CHH family (i.e. MIH and CHH) in reproduction in penaeid shrimps have also been reported. Thus crustacean reproduction is an end result of multiple vitellogenic related endocrine cascades (Figure 4). Nevertheless, the bi hormonal axis is still central to shrimp reproductive endocrinology.

The neural portion of the decapod eyestalk is an extension of the brain (supra-esophageal ganglion). A group of cell bodies usually found as faint blue white in live specimens is located in the middle portion of the medulla terminalis is termed as X organ. At least eight neuro-hormones appear to be synthesized in the X organ and it contains about 150 -200 neurosecretory cells. The neuro hormones produced in these cells are transported via axon and ends in the blood sinus called the sinus gland in the medulla externa (Figure 5). These hormones regulate several physiological functions, for example, gonad activity, molting, and blood-sugar level. cHH or crustacean hyperglycemic hormone family are structurally related neuro-hormones of X-organ. Two sub types of cHH peptides are recognized type 1 and type 11; type 1 peptides are cHH sensu stricto are typically 72 amino acids. Their protein precursor contains cryptic peptide, cHH precursor related peptide (CPRP), between the signal peptide and cHH progenitor sequence. This type 1 cHH is named because upon injection, it elicits hyperglycemia in animals. Type 11 peptides are not with CPRP, and it contains three neuro-hormones: Gonad/vitellogenesis inhibiting hormone (G/VIH), Molt inhibiting hormone (MIH) and Mandibular organ inhibiting hormone (MOIH).

Gonad/vitellogenesis inhibiting hormone:

G/VIH actively participate in ovarian development and is a key hormone for the reproduction in crustacea. It is believed that GIH is more intense than any other hormone in the crustacea. Gene coding for GIH has been characterized and cloned from several crustaceans: For example, terrestrial isopod (*Armadillidium vulgarae*), lobsters (*Homarus americanus H. gammarus* and *Nephropse norvegicus*) and shrimps (*P. monodon, Metapenaeus ensis, Litopenaeus vannamei*), prawn (*Macrobrachium nipponnese*) and deep sea shrimp (*Rimicaris Kairei*). GIH is expressed in both males and females; it has been expressed in tissues such as eyestalk and brain. The presence of GIH has also been found in larval crustaceans as well. The expression of GIH mRNA is found to be lower in the immature stage, however, it was found to be higher in previtellogenic stage.

Mating

After sexual maturity, a female is typically inseminated by males when each time she molts in the case of closed thelycum species. However, in open thelycum species such as P. vannamei, males mate with only females with a ripe ovary, and towards the end of the molt cycle. Mating behaviour is triggered by photoperiod and occurs during sun sets. It is suggested the involvement of an external hormone (=pheromone) in attracting the males. Courtship starts when a male approaches a female and attempts to get underneath her from behind. The female then swims away and is followed by the male. This chasing behaviour can occur for several minutes, and more than one male may be involved. Males often are observed chasing immature females as well as other males. During insemination, the male briefly turns upside down while remaining parallel to the female and grasps her from underneath with his pereopods for one or two seconds, at which time spermatophore transfer occurs.

Most penaeid species have a complex life history; in all, the penaeids, egg hatches to pelagic larvae, which is entirely distinct from juveniles and adults in morphology and habits. Unlike adults, these larvae float in the water column and feeding up on co-existing plankton organisms. This biphasic life cycle is considered to be an old evolutionary trait, and it forms a major input to the biodiversity and productivity of the marine ecosystem. In all cultivable penaeid shrimps, three forms of larvae are distinguished: Nauplius, Protozoea (=zoea) and mysis. These larval forms are distinguished based on the presence or absence of locomotory appendages. The first larval forms of penaeid shrimp are the nauplius, and it is the most ancestral larval form. In decapods, it occurs only in the dendrobranchiate group where penaeid shrimps are included. In all other decapod groups this phase is embedded in the embryonic phase. The major morphological characteristic of this group is the absence of thoracic somites (segments), and locomotion is exclusively carried out by cephalic (head) appendages. In penaeids this stage is a non-feeding stage. The nauplius swims upwards, attracted by the light on the surface of the sea. It has no mouth or alimentary canal and hence cannot feed. It grows utilizing the yok stored in its body and moults five times before metamorphosing into the next larval stage, the protozoea, after 36-48 h. The protozoea are filter feeders feeding on the microalgae. There is three protozoeal stages that last for 3-4 days before metamorphosing into the next larval stage, called mysis. There are there mysis stages which are also filter feeders. The mysis stage metamorphoses into post larval stage after molting in 3-4 days. The post larvae lose filter feeding habit and become carnivorous feeding on the small planktonic animals. The post larvae look like a miniature adult and settle at the bottom after 4-5 days of planktonic life. The transition from post larvae to juveniles is gradual after many moults and days, usually 20-30 days.

Molting and growth: Body of the crustaceans are covered with a rigid exoskeleton. They periodically shed their old exoskeleton in order to facilitate metamorphoses (in larval stages), growth (in post larvae, juveniles and sub adults) and reproduction (in adults). This process is called moulting or ecdysis, and it is a consequence of cyclic mopho-physiological events. The most obvious manifestation of molt cycle is the shedding of the exoskeleton and it comprise only few minutes. The vast majority of the events related to molt cycle occur internally with subtle morphological alterations. In vannamei, by analysing the aspect of cuticle, epidermis and moult processes of uropods, 5 major moult stages were defined: early- and late post-moult (A and B), inter-moult (C) and earlyand late pre-moult (D1 and D2). Briefly, the main characteristics used to discern the stages were A: epidermal tissue is present inside the setal lumen; B: the epidermis is retreating from the setae but is still present in the base of the setae; C: the epidermis lies on a line just underneath the base of the setae; D1: apolysis causes a translucent space to form between the old cuticle and the epidermis; D2: the new, folded cuticle and the new setae have become visible; E: ecdysis, the shedding of the old moult skin. As E stage lasted only few minutes, the moult was considered as the transition from D2 to A and was not further included in the analysis. In juveniles total molt cycle is ranged between 5 and 6.5 days.

Penaeus vannamei aquaculture: Biological characteristics and essential principles of culture management

Panigrahi, A., Vinay, T. N and Balasubramanian, C. P

Overview and production status

In India, shrimp aquaculture was synonymous with *P. monodon* culture, and its growth rate of the sector was phenomenal till 1995. Since 1995, the sector has been plagued by viral diseases, especially White Spot Syndrome Virus (WSSV). In 1997, the Ministry of Agriculture established the Aquaculture Authority under the Environment Protection Act, 1986. In 2005, From 2000 onwards there was a gradual increase in production which reached a maximum of 1,40,000mt in 2006-07. But in 2007-08 and 2008-09, the production levels reduced drastically and reached the pre-1995 level of 75,000mt. The introduction of *Litopenaeus vannamei* in 2009 led to the recovery of the sector, with the total production levels reaching 2,20,000mt in 2011-12, which further reached a level of 8,15,000 tonnes from a total area under culture of around 1,08,000 hectares in 2020-21 (Source: MPEDA) marking sea food export more than 5 billion dollars. The total volume of farmed crustaceans in India in 2021 was 8.8 lakh tonnes, of which farmed shrimps alone accounted for 8.42 lakh tonnes (FAO, 2019). Recently, in continuation of the phenomenal growth, a production of 8.42 lakh tonnes of shrimp was recorded in 2020 comprising of mostly pacific white shrimp *P. vannamei* and rest tiger shrimp *P. monodon*. Out of total 6,15,000 tonnes exported shrimp, 80,000 tonnes are wild caught, 15,000 tonnes black tiger and 5,20,000 tonnes of Pacific white shrimp. In the present rate of up-scaling, a significant increase in production up to 1.1—1.3 million tonnes by 2022.

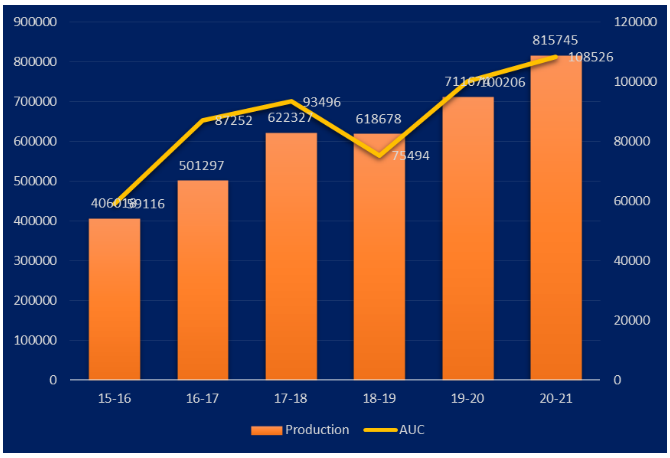


Fig. Shrimp production status

However, farming activity in the entire country sometimes enters into troubled water because of the market price fluctuation and disease outbreaks like sporadic WSSV, White Faeces Disease, EHP, Slow growth, Running mortality syndrome. The production performance dipped and many farmers chose to suspend the farming activity till a better weather. In other front, farming in low saline water are encountering fewer problems with better productivity and profitability. Again, tiger shrimp farming is getting attention and coming back in low density with a growth of 30g in 120 days.

Time line of Vannamei introduction

The SPF/ SPR lines of brood stock and culturing it under a total bio secured system become the panacea for the aquaculture of the shrimp industry. The Pacific white shrimp *Penaeus vannamei*, a species native to Latin America, was taken up for culture in some of the Asian countries around the turn of the century. These white shrimp have many advantages like tolerance to a higher range of stocking densities, high growth rate, crowding tolerance, a wider range of salinities, lower temperatures, lower-protein food requirements, and captive breeding, leading to its commercialization in America and Asia. Further, a most important attribute of *P. vannamei* is successful captive breeding, which produces genetically superior and high health seeds. Captured wild seeds were used in Latin America for extensive pond culture of *P. vannamei* until the late 1990s. It was introduced to Pacific islands in the early 1970s, and in 1996, it was introduced in China and subsequently in other coastal Asian counties from 2000-01. Introduction of *P. vannamei* in Indian aquaculture through SPF broodstocks in 2009 opened up a new vista in coastal aquaculture of the country. However, worldwide shrimp farming activity is plagued by diseases and unsustainable practices, which sometimes affect the coastal ecosystem and livelihood.

Indian government has shown great caution due to the potential risks involved, thus carried out a risk assessment and then finally allowed official import of certified SPF brood stock with such introductions, despite interest in the species due to its perceived tolerance to WSSV when SPF integrity is maintained with uncompromised farm biosecurity.

Genetic improvement program

Life Cycle and Reproductive Biology

P. vannamei has a complicated life cycle consisting of an offshore oceanic and inshore estuarine phase associated with spawning and juvenile growth, respectively. In nature, they spawn in sea after successful mating**.** When the eggs are released from the body, they pass the sperm and get fertilized. *P. vannamei* weighing 30–45 g will spawn 100 000–250 000 eggs of approximately 0.22 mm in diameter. Floating eggs, 200-300 microns in diameter, drift with the sea currents and develop into nauplii. Hatching occurs about 16 hours after spawning and fertilization. The next larval stages (protozoea, mysis and early postlarvae, respectively) remain planktonic for some time, eat phytoplankton and zooplankton, and are carried towards the shore by tidal currents. The larval stages, starting with the zoea are filter feeders. The postlarvae (PL) change their planktonic habit about 5 days after moulting into PL, move inshore and begin feeding on benthic detritus, decaying plant material, plankton, worms, bivalves and crustaceans. This species is one of the open thelycum species, and they differ from the closed thelycum species in the mating pattern. In open thelycum species, mating takes place when the female shell already has hardened. The spermatophore is visible as a coarse whitish mass of approximately 1 x 1 cm, glued to the female thelycum. In *P. vannamei* the color of the ovary changes from yellow to orange just before spawning; this can be used as a ripeness indicator.

Domestication and selection program

Domestication and genetic selection programmes then provided more consistent supplies of high quality, disease free or resistant PL, which were cultured and reproduced. The selective breeding program in Hawaii and other places from 1989 resulted in the production of SPF and SPR lines, leading to the industry in the United States of America and Asia. Domestication and genetic selection programs for reproduction have paid a rich dividend in ensuring consistent high-quality, disease-free or resistant PL supplies. Pacific White Shrimp Broodstock is selectively bred for good maturation performance, fast growth, resistance to diseases, high survival to the Hatcheries, and generation after generation, adding to the vigour. Now, the specific pathogens free (SPF) or resistant (SPR) lines with superior and high health seeds are available in Hawaii and other places. *L.vannamei*, selected for growth performance, has heritabilities of 0.15-0.3, meaning that the selected breeders produced offspring that grew 15-30% faster than the parents. With *L.vannamei*, it was shown that domesticated stocks performed better than wild stocks. In fact, research work by some state and private companies has focused efforts on the development of SPF strains that are also resistant to specific pathogens (SPF/SPR). These strains are typically resistant to only one pathogen, currently largely either TSV or IHHNV, but some work has indicated that strains with multiple resistances to TSV and WSSV (at up to 25 per cent survival to challenge tests) may be possible. This is accomplished by challenging sub-lots of shrimp families to a particular pathogen (or combination of pathogens) and then selecting the most resistant families as broodstock for the next generation. This negative correlation between growth and disease resistance (as it is found often like mean family survival to a TSV challenge) must therefore be taken into account. A US-based facility producing SPF and SPR *P. vannamei* has reportedly achieved a growth rate potential of 2g/week with families of shrimp selected for resistance to TSV, with no negative correlation between growth and survival.

Other specific advantages of genetically improved variety include rapid growth rate, high stocking density, tolerance of low salinities and temperatures, lower protein requirements (and therefore production costs), disease resistance (if SPR stocks are used), and high survival during larval rearing. SPR strains of shrimp, however, do not necessarily have to be SPF. Latin America is now almost exclusively using pond-grown and (often) disease checked and quarantined SPR *P. vannamei* due to their better performance in maturation, hatcheries and grow-out ponds. At that stage, depending only on the imported broodstocks may not be enough and economical. Therefore, India should have its own selective breeding program and multiplication centre to produce SPF seeds. Recently, Rajive Gandhi Centre for Aquaculture (RGCA) has tied up with M/s. Oceanic Institute, Hawaii, pioneers in the domestication of *P. vannamei* in the world for aquaculture at TASPARC (The Andhra Pradesh Shrimp Seed Production, Supply and Research Centre) facility of MPEDA at Vishakhapatnam in Andhra Pradesh. With the germplasm (Postlarvae) provided by the collaborating institute, a targeted high quality SPF broodstock of 4500 nos can be supplied to the growing industry.

SPF and SPR Broodstock

These specific pathogen free (SPF), Specific pathogen resistant (SPR) or high health stock is one of the best ways to recruit the stock in the hatchery. SPF broodstock is sourced by approved hatchery operators from approved international suppliers for seed production and supply to farmers. Hatcheries normally prefer wild broodstocks due to the belief that they produce more and healthier nauplii. However, in recent years, the high risk of introducing viral pathogens with wild broodstock has changed this preference. Domesticated stocks either genetically improved or suspected to be resistant or tolerant to specific pathogens may be used as broodstock. SPF stocks are generally maintained in highly biosecure facilities and their offspring (designated "high health" rather than SPF) are supplied to the industry. SPR shrimp are not susceptible to infection by one or several specific pathogens, and Specific Pathogen Tolerant (SPT) shrimp are intentionally bred to develop resistance to the disease caused by one or several specific pathogens.

The SPF stock of *P. vannamei* that is imported should be free of the following two sets of pathogens:

A. Pathogens exotic to India and likely to cause damage to *P. monodon*

1. Taura syndrome virus (TSV)

2. Yellowhead virus (YHV)

3 .Hepatopancreatic parvo-like virus (HPV)

4. *Baculovirus penaei* (BP)

5. Infectious myonecrosis virus (IMNV)

6. Necrotising hepatopancreatitis bacterium α-Proteobacterium (NHPB)

7. Necrotising hepatopancreatitis (NHP)

B. Pathogens reported from *P. vannamei* and which cause mortality or retarded growth/Pathogens not exotic to India and need to be avoided, so that introduced *P. vannamei* performs well

1. White spot syndrome virus (WSSV)

2. Infectious hypodermal and haematopoietic necrosis virus (IHHNV)

3. Monodon Baculovirus (MBV)

4. *P.vannamei* nodavirus (PvNV)

Biosecurity principle and protocols

The role of biosecurity measures is to diminish the risks arising from the entry, establishment or spread of pathogens within the system to a manageable level. Biosecurity has been defined as "*...sets of practices that will reduce the probability of a pathogen introduction and its subsequent spread from one place to another...*" (Lotz, 1997). Biosecurity protocols are advocated to be followed in all coastal aquaculture units to minimize the disease risk (Lightner, 2011)and become very important when viral /bacterial threat is imminent and moreover when specific pathogen free or specific pathogen resistant stocks are used (as in the case of *P. vannamei*).

The basic elements of a biosecurity programme in a shrimp hatchery include the physical, chemical and biological methods necessary to protect the hatchery from the consequences of all diseases that represent a high risk. 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 appropriate level of biosecurity to be applied will generally be a function of ease of implementation and cost relative to the impact of the disease on the production operations (Fegan and Clifford, 2001). There are two common ways by which disease transmission occurs:

•**Vertical transmission** - from mother shrimp to the post larvae in hatchery systems

•**Horizontal transmission** - from one affected shrimp to the other in farming systems.

The components of biosecurity in an aquaculture facility comprises

i) **prevention**- protection of the cultured/managed organisms (especially pathogens) and the protection of humans and ecosystems from the adverse effects of the introduced culture system, and its targeted and non-targeted organisms, ii) **control**- control of the culture system, the movement of organisms, risk related activities, and monitoring and recording of actions taken and iii) **contingency planning-** planning for all possible eventualities. The biosecurity issues in shrimp hatcheries may be either **internal** concerning the introduction and transfer of pathogens within the facility or **external** concerning the introduction and transfer of pathogens from outside sources to the facility or vice versa. In case of disease outbreak within the aquaculture facilities, the options available are either **treatment-** by application of methods that reduce the effects of the diseases, **containment-** by restriction of the disease from spreading to other tanks/facilities, or **elimination-** of the diseases from the vicinity. Implementation of a biosecurity programme for a shrimp hatchery should include the following elements:

The physical separation or isolation of the different production facilities is a feature of good hatchery design and should be incorporated into the construction of new hatcheries. In existing hatcheries with no physical separation, effective isolation may also be achieved through the construction of barriers and the implementation of process and product flow controls. Water distribution and discharge from the hatchery should also be compatible with all biosecurity protocols. The seawater to be disinfected with hypochlorite solution (20ppm active ingredient for not less than 30 minutes) and inactivated. Vehicle tyre bath, Footbaths containing hypochlorite solution at >50 ppm active ingredient) and hands (bottles containing iodine-PVP 20 ppm and/or 70% alcohol) to be used upon entering and exiting the unit. All wastewater must be collected into another tank for chlorination (20 ppm for not less than 60 minutes) and dechlorinating before release to the environment. All mortalities or infected animals must be incinerated or disposed of.

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

- Specific pathogen free (SPF) or high health (HH) shrimp stocks should be used.
- All the incoming stocks should be quarantined in the designated area.
- All incoming stock should be analyzed for diseases.
- All incoming water sources should be treated to eliminate pathogens.
- Equipment and materials should be sterilized and maintained clean.
- Personal hygiene measures including washing of hands and feet and clothing.
- Knowledge of the potential pathogenic diseases and the sources of risk and methods and techniques for their control and /or eradication
- Maintenance of optimum environmental conditions.
- Immune enhancers and probiotics to be used in place of antibiotics.

The following SOPs should be strictly followed to prevent the horizontal transmission of viruses in the culture ponds:

Elimination of viral particles from soil through drying and disinfection, Using quality water treated and filtered, Elimination and prevention of vectors entry, Prevention of viral pathogens in feed, Prevention of viral pathogens through birds and animals, Following strict sanitary protocols should be followed by all farm workers and disinfection of the feet and hands of the farm workers is mandatory before entering any pond, Disinfection of all the pond implements before use in any pond, Avoidance of fresh feed or farm made semi moist feed, Following proper disinfection protocol in case of disease outbreak or emergency harvest.

Preventing introduction, spread and increase resistance to disease and eradicate it if it occurs (hatchery/farm) is the essential principle of biosecurity which comprises proper engineering designing, broodstock screening or SPF/SPR broodstock and quarantine measures, monitoring health and protection of health, prioritizing and evaluating risks and critical control points, mitigating harmful diseases and other risks through ideal biosecurity plans and programs including biosecurity check list and financial dividends

Quarantine facility and protocols

SPF *L.vannamei* brood stock is to be imported into India only with a valid sanitary import permit through the designated port of entry and strictly following the guidelines for operation of aquatic quarantine facilities to import SPF *P. vannamei*. Before passing to the production system, the broodstock must be screened for pathogens (i.e. via dot blot, polymerase chain reaction (PCR), immunoblot etc.). Hatchery unit should comply with the set standards as propagated by CIBA and set by CAA. The quarantine facilities are essentially a closed holding area where shrimp are kept in individual tanks until the results of screening for viruses (and for bacteria, where applicable) are known. The broodstock quarantine unit should be physically isolated from the rest of the hatchery facilities. The quarantine unit should have the following characteristics:

- ✓ Adequate isolation from all of the rearing and production areas to avoid any possible cross contamination.
- ✓ Should be in an enclosed and covered building with no direct access to the outside.
- ✓ There should be means provided for disinfection of feet (footbaths containing hypochlorite solution at >50 ppm active ingredient) and hands (bottles containing iodine-PVP (20 ppm and/or 70% alcohol) to be used upon entering and exiting the unit.
- ✓ Entrance to the quarantine area should be restricted to the personnel assigned to work exclusively in this area.
- ✓ Quarantine unit staff should enter through a dressing room, where they remove their street clothes and take a shower before going to another dressing room to put on working clothes and boots. At the end of the working shift, the sequence should be reversed.
- ✓ An adequate number of plastic buckets should be available in the quarantine room to facilitate effective daily routine movement of shrimp in and out of the facility.
- ✓ The quarantine facility should have an independent supply of water and air with separate treatment and disinfection systems and a system for the treatment of effluents to prevent the potential escape of pathogens into the environment.
- ✓ The seawater to be used in the facility must enter a storage tank where it will be treated with hypochlorite solution (20 ppm active ingredient for not less than 30 minutes) before inactivating with sodium thiosulfate (1 ppm for every ppm of residual chlorine) and strong aeration.
- ✓ All wastewater must be collected into another tank for chlorination (20 ppm for not less than 60 minutes) and dechlorination before release to the environment.
- ✓ All mortalities or infected animals must be incinerated or disposed of in another approved manner.

- ✓ Used plastic containers and hoses must be washed and disinfected with hypochlorite solution (20 ppm) before reuse.
- ✓ All the implements used in the quarantine unit must be clearly marked and should remain in the quarantine area. Facilities for disinfection of all equipment at the end of each day should be available.

Health Management aspects

Health management becomes very crucial, especially as many viral and bacterial diseases are reported in this species. The seed being one of the major sources of this virus and vertical transmission of the virus has been established, the hatcheries have to adopt many precautionary principles. From hatching, it takes about 19-20 days to reach harvest at PL-10. Care is taken to reduce bacterial/pathogen contamination of the larval facilities using a combination of periodic dry-outs and disinfections, inlet water settlement, filtration and/or chlorination, disinfection of nauplii, water exchange and the use of antibiotics or (preferably) probiotics. Biosecurity measures must be implemented for each phase, Water treatment, high quality feeds (both live and inert) and Rapid diagnostic methods for field use and expertise are required to use it. Live feeds should be proven free from pathogens or treated suitably to kill them. Eggs and nauplius should be washed and cleaned using appropriate disinfectants to minimize "vertical" transmission of pathogens from broodstock to larvae. Only healthy nauplii should be selected.

The most successful strategies for controlling diseases in shrimp ponds are based on a combination of prevention by exclusion and Better Management Practices that focus on creating a healthy, non-stressful environment for the shrimp. *P. vannamei* SPF status of the broodstock and disease free seeds are to be procured, and for this reason, the biosecurity of the farm should be intact at all point of time. The gut content colour is a good indicator of the probable health status and corrective action to be taken. Black/brown/green gut content implies underfeeding, 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. Testing periodically for any disease prevalence like for WSSV and IHHNV during the culture is required to understand and act as per the infection presence.

In case of any sick or dead shrimps- gills, gut content of the shrimp, water quality and pond bottom condition can be checked, In case of wsd-informing neighbours; and if mortality is increasing over 2 days don't letting the water to change, in case. If >50% of the shrimp are not feeding, harvesting can be considered without draining the pond. *The options to deal with the disease problems includes*-Immunoprophylaxis, Other novel therapeutic strategies (RNA interference approach), and integrated eco based management

Technology Up gradation

Initial five/ six year of vannamei adoption went on smoothly and successfully, location specific fine-tuning is required. This species is found to be well suited to Indian conditions, and being amenable for high stock densities, a higher productivity can be obtained. In this connection, reduction in the cost of production is the priority especially taking the indigenous culture and feed technology further. Following are some of the priority areas for research in *P. vannamei*.

- Multiplication centres of *P. vannamei* should be encouraged, but a mechanism to ensure Pathogen free status of the stocks imported/held/sold, should be put in place.
- Low larval survival rate in India compared to that obtained worldwide
- Robust disease surveillance during hatchery operations with special emphasis on problems like Zeoa2 syndrome
- Initiation for the establishment of Multiplication centres in India and related research on selective breeding program to avoid monopolistic situation of broodstock supply
- Continued development of SPR lines of *P. vannamei* for viruses including TSV, WSSV, IHHNV, BMNV and IMNV.
- Development of faster growing and customized lines of SPF/SPR stocks.
- Effective disinfection procedures for eggs, nauplii and PL in hatcheries.
- Adequate replacements (i.e. probiotics and immunostimulants) for antibiotics.
- Vaccination and other effective treatments for shrimp viruses.
- Replacement of non-eco-friendly and costly marine meals in shrimp feeds.
- Efficient water treatment and management systems for closed culture systems.

Conclusion

Embracing “Good Aquaculture Practices” is the best way to address aquaculture's environmental and sustainability concerns. It is also imperative to establish an audit and certification system has to be set up in India. Implementation of a biosecurity measures by shrimp hatcheries and farms will ensure restriction of the disease from spreading to other facilities or elimination of the diseases from the vicinity of the aquaculture facility. Misconception like “SPF seed never get affected by virus” should be removed. However, keeping the requirements and future projection in mind, it is worthwhile to mention here that India should start a domestication program to produce a genetically improved *P. vannamei* through multiplication centre should be compiled. A national Aquaculture Certification Standards for India and HACCP guidelines compliance by the farming sector will boost India’s attempt towards sustainable aquaculture. In the pretext of evolving culture practices and introduction and proliferation of farming of new species, evolution of new culture practices like zero water exchange and/ or probiotic/biofloc/periphyton based farming is to be encouraged.

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Site selection, Design, and Construction of Shrimp hatchery

Jayanthi, M

Introduction

Development of seed production technology and the establishment of hatcheries have preceded large scale developments in commercial production of shrimps. Hatchery design is important to produce healthy seed and to attain the required production target. Requirements of the hatchery design depend on species type, production aimed and level of financial inputs. Species should be identified before the design and production target can be determined based on market demand and financial capacity. Hence, the hatchery technology should provide the larvae with the most conducive environment similar to that of the sea. This involves proper site selection and designing of the hatchery taking into account the requirements of the species to be reared.

A basic prerequisite in the design and construction of a hatchery is an understanding of the biological requirements of the species and the concept of different systems involved in the hatchery. The common operations in the shrimp hatchery are broodstock maintenance, induced maturation (shrimps), spawning/ hatching larval rearing, live-feed culture (phytoplankton/ zooplankton) and post-larval rearing. Generally, the size of a hatchery should be based on its functional requirements and economic efficiency. Based on the level of operation, production output and financial investment, hatchery practice can be broadly grouped into three categories viz: small-scale, medium-scale and large-scale hatchery. The requirements will vary based on the size and most of the hatcheries operating at commercial scale are large ones. The major characteristics of each group are shown in Table 1.

Size of the hatchery

Small-scale hatchery

It is a "backyard" hatchery usually owned and managed by the shrimp grower himself using family members or immediate relatives for additional labor. The main goal is to supply his own shrimp farm with the required number of fry and the excess may be sold to neighboring growers. Usually, the hatchery site is an extension of the farmhouse with floor space ranging from a few square meters up to about 1000 square meters. In such hatchery operation, the total production capability seldom exceeds 5 million postlarvae per annum and the hatchery is operated by not more than 2 technical personnel.

Medium-scale hatchery

It is relatively larger than the small-scale hatchery in terms of capital investment, hatchery size, production capability and scale of operation. While hatchery management is somewhat similar to that of a small-scale hatchery, the production capacity range between 10–20 million postlarvae per annum and is operated by about 3 technical personnel and 3–4 laborers. This type of hatchery is usually put up by small cooperatives to supply the required shrimp fry to its member growers. Private entrepreneurs or government agencies may also establish hatchery of such operational scale to produce fry of sale or distribution to the growers.

Large-scale hatchery

This scale of the hatchery is commercially run by big corporations, national agencies or cooperative projects. While the capital and operational investment far exceed that of the medium-scale hatchery, the production capability of such hatchery usually exceeds 20 million postlarvae per annum. Such hatchery is centrally and systematically managed and is supported by a pool of not less than 6 technical personnel and 6–10 laborers

Table 1. Criteria for classification of shrimp hatcheries

Item		Small	Medium	Large
1.	Ownership and operation set-up	Family members serve as hatchery workers	Small cooperative	Big corporations, national agencies
		Fry for self-use	Supply fry to members	Fry for commercial purposes
2.	Area or extent covered	Usually using the backyard area	2000–5000 sq.m.	5000 sq.m. to l ha.
3.	Amount of production	1–5 million/year	10–20 million/year	More than 20 million
4.	Number of employees or technicians	1 technician, 2 workers	3 technicians, 3–4 workers	3–6 technicians 6–10 workers
5.	Total tank capacity	20–100 tons	100–1000 tons	More than 1000 tons

Site selection criteria for shrimp hatchery

The hatcheries are to be located close to the seawater source. The major criteria for selection of the hatchery site are discussed below.

Seawater quality :

The foremost requirement of the hatchery is clean seawater. The seawater should not be turbid and free from suspended solids. Heavy winds and wave action and inflow from rivers/creeks are the major causes for the turbidity in the nearshore areas. Ideal sites are those that are located near calm sea coast away from any freshwater/brackishwater inflow.

Water temperature

Temperature plays a very definite role in the growth of larvae. Seawater temperature between 25^{o}C to 32^{o}C is optimal for most of the tropical species of shrimp. Hence, the hatchery should not be located in areas where there is severe winter or summer.

Salinity

It is one of the most important factors controlling the physiology of marine organisms. The optimal salinity for the growth and survival of shrimp is between 30 and 34 ppt, though the adults could tolerate a wide range of salinity. Heavy rainfall and freshwater influx from rivers reduce the salinity of the seawater and make it unsuitable for use in the hatchery. Hence, the hatchery should not be located in the river mouth or in areas that experience heavy rainfall.

While selecting the site, it should be seen that all the above parameters of seawater are within tolerable levels. An ideal site should have optimal levels of these parameters during the major part of the year.

Weather

The weather conditions of the site have a profound influence on seawater quality. Heavy monsoon rains will seriously affect the salinity and turbidity of the seawater, while severe summer or winter will lead to high or low-temperature conditions. Similarly, cyclone-prone areas should also be avoided since it would cause severe damage to the hatchery installations.

Nearness to a shrimp landing site

Hatcheries generally depend on the wild-caught broodstock or spawners of shrimps. Hence, it is essential that the hatchery is located nearer to the shrimp landing centres so that long-distance transportation of the broodstock/ spawners could be avoided. Transportation of mature shrimp results in serious stress, which could lead to the regression of the ovaries or death of the shrimps.

Infrastructure facilities

Availability of infrastructure facilities such as approach roads and electricity is one of the important considerations for selecting a site. The hatchery operation essentially depends on electrically operated machinery such as water pumps and air blowers. Complete dependence on the generator is not advisable since the operational costs will be very high. Similarly, without road and communication, a hatchery may not be able to function since all the material inputs have to be brought in from outside. Hence, a site with the proper road, communication facility, and state-sponsored electric supply should be chosen.

Physical facilities required for the hatchery

In designing a hatchery, ample space should be provided for the rearing and support facilities needed in operation. The most important design criteria in sea water-based hatcheries are the materials to be used. Materials, which do not corrode in the saline environment, should only be used. Plastics, PVC, concrete and wood are the materials, which are commonly used in these types of hatcheries. A functional hatchery should have the following essential components

1. Seawater supply system
2. Air supply system
3. Tanks
4. Buildings
5. Effluent water treatment system

Two major issues have to be considered for the construction of hatcheries for *Litopenaeus vannamei* - i) Risk of pathogen transmission and ii) stress-free environment.

In the operation of hatcheries, several measures have been suggested to ensure the prevention of pathogen transfer and water treatment to overcome these issues. But it is better to ensure that the location does not increase the risk of pathogen introduction and contamination with pollutants. The following additional site selection criteria are required to be followed for a successful healthy and disease-free shrimp seed.

Site Selection Criteria to avoid disease risk

Disease-causing pathogens could emanate from live fish/shrimp, so care should be taken to avoid locating the hatchery in such areas. Shrimp hatcheries located close could be releasing the wastewater without treatment and increase the risk of contaminating the source water. Though water treatment protocols are used, the risk increases with the level of contamination. Hence there should be a distance of at least 200 m as a buffer zone between the hatcheries. Similarly, sea-based shrimp farms releasing their wastewater into the sea will be a major risk factor. Hatcheries should be located 200 m away from such farms.

If hatcheries already constructed are located within a very close range of less than 200 m from other shrimp hatcheries/farms, the intake water treatment system and the Wastewater Treatment system should be improved with two days retention period, which will necessitate double the capacity of the tanks normally used. Other live fish handling centers like fish landing centers, fish drying centers and fish processing plants should not be located within 500m of the shrimp hatchery. Site selection criteria to avoid the risk of contamination with pollutants. Heavy metals, pesticides, radioactive materials, ammonia, nitrite, and nitrate are the major contaminants that affect the shrimp larval survival. The following guidelines need to be followed to avoid these contaminants.

Avoid locating hatcheries near an industrial belt where the wastewater is being released into rivers/hatcheries. Keep a distance of 1 km from any factory located on the sea coast. This is to be strictly followed in the case of fertilizer plants, atomic power plants, and thermal power plants. Hatcheries should be 500m away from important coastal ecosystems such as mangroves, bio reserves, etc., to protect the coastal ecosystems. Areas where heavy agricultural run-off is expected should also be avoided.

Hatcheries should not be located in areas with high tidal impact and wave actions since this will create a very high level of suspended matter, and water treatment will become a costly affair. Because of the same reason, we should not select areas near river/estuarine mouths

Site selection criteria for avoiding stress to the environment

Quality shrimp seeds could be produced only when they are reared under stress-free optimal conditions. The primary considerations are pollution and fluctuations in seawater quality during the culture period. The important requirements are temperature, salinity, pH, Ammonia, and presence of contaminants. The following optimal conditions should be available at least for 8-10 months in a year to have a successful hatchery operation. At the time of site selection, the source water should be tested, and in case if any of the parameters are widely varying, then the site should be avoided.

Ideal range for water quality parameters in maturation/hatchery facilities

Parameter	Ideal range
Salinity	29–34 ppt
PH	7.8–8.2
Temperature	28–32 oC
Oxygen	> 4 ppm
Heavy metals/pesticides minimal level	
Ammonia (NH3)	<0.1 ppm
Nitrite (NO2)	< 0.1 ppm
Nitrate (NO3)	<10 ppm
Hydrogen sulphide (H2S)	<0.003 ppm
Chlorine residue	< 0.01 ppm
Cadmium	<0.005 ppm
Chromium	<.025 ppm
Copper	<0.003 pp m
Iron	<0.3 ppm
Mercury	<0.0001 ppm
Manganese	<0.05 ppm
Nickel	<0.05 ppm
Lead	<0.05 ppm
Zinc	<0.05 ppm

Water holding tanks

The tanks in the hatchery are named as to the purpose for which they are used, i.e., reservoirs, overhead tanks, maturation tanks, spawning tanks, larval rearing tanks, nursery tanks or postlarval rearing tanks and algal culture tanks.

The materials of construction vary from site to site and on the availability of materials. Depending on the scale of operation and on planned longevity of the facilities, the tanks may be any one of the following:

a) Plastic lined with Aluminium frame: Usually used for small scale operation; longevity minimal,
b) Fibreglass Reinforced Plastics: Portable, longevity high; can be used for small scale operations,
c) Concrete hollow blocks: Cheap and easy to install; Permanent installation; Large-scale operations, and
d) Reinforced concrete: Expensive than hollow blocks; permanent installation; large-scale operation.

Tanks made of concrete should be coated with epoxy paint to provide a smooth interior surface. This would prevent the leaching of harmful chemicals into the tank and discourage the growth of pathogenic organisms, which could flourish in the crevices of unpainted surfaces. All angled parts of the tank should be rounded off to facilitate cleaning and minimize `dead spots' in the tank.

The commonly used **shape** of tanks is rectangular, circular or oblong. Rectangular tanks are preferable for permanent installations, where the scale of operation may be expanded from existing facilities.

Reservoirs: to provide the water needs of the hatchery during times when the pump is not in operation or when the water demand is much greater than the pump capacity over a relatively short period; are used for the chemical treatment of the seawater; capacity of the reservoir depends on the nature of operation in the hatchery, pump capacity, the treatment used and the duration of the treatment.

Overhead tank: to maintain continuous water supply to the different tanks by gravity; without an overhead tank, water supply to individual tanks will necessitate secondary pumping; capacity of the overhead tank and its height depending on the actual water required per day, pump capacity and the dimensions of the pipeline.

Maturation tanks: water capacity of 5 - 10 m^3 with 1 m effective water depth suitable; to be housed in closed sheds under darkness to avoid disturbances from human movement.

Spawning tanks: 250 - 500-litre capacity.

Larval and postlarval rearing tanks: 10 - 50 m^3 capacity with effective water depth ranging from 1 - 1.5 m; should be housed in a shed with reduced light conditions to prevent heavy growth of algae.

Algal culture tanks: 1 - 10 m^3 capacity depending on the scale of hatchery operation and the daily requirements; effective water depth should not be more than 1 m to allow light penetration through the whole water column; Tanks with the capacities of 5 - 10 m^3 more suitable for small scale operations and tanks of higher capacities for large scale operations.

Hatchery Building and other facilities :

In our sub-tropical conditions, the hatchery building need not be as elaborate as those located in temperate areas. The building need not be totally enclosed. Roofing is necessary to shield the larval rearing tanks from direct sunlight and rain. Walls may be necessary for areas where there is heavy cold draft wind.

The building should provide space for the living quarters of technicians, a phycology laboratory, a feed preparation room, a laboratory for water quality and biological analysis and a packing room. Pumps and filtration units on the seaward side and blowers and generators on the landward side should be located in separate buildings.

The layout of the various units of the hatchery should be located in such a way that there is an ease of movement, optimal use of water and air supply system. A model layout is presented in Fig. below.

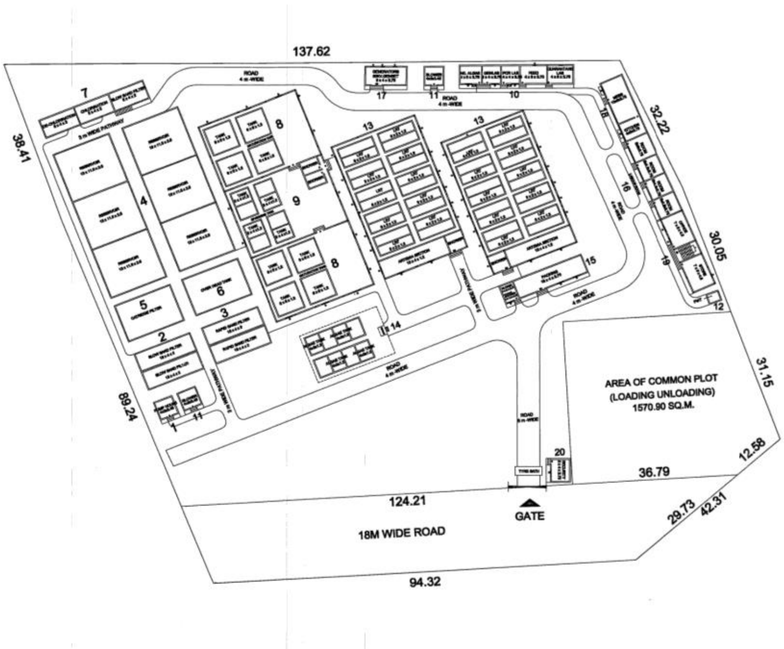

Fig. Hatchery layout and design model

Water intake, treatment and RAS in shrimp hatchery

Nila Rekha, P

Introduction

The shrimp hatchery technology has advanced over the decades, but hatchery production is more often hampered by severe mortalities due In many instances, it has been observed the water source and the supply system in the hatchery has been the cause for some infections leading to mass mortality of the shrimp larvae. The shrimp hatchery water supply system usually consists of modern technology (mechanical filtration, chemical treatment, UV filtration, ozonation, etc.) to make the water acceptable.

Seawater supply system

The seawater supply should be clean and free from pollutants. If the water becomes turbid, it should pump to a sedimentation tank to allow suspended solids to settle, and only upper layers of clean water must be pumped into filter tanks. Water is pumped to filter tanks, stored in the reservoir and distributed by gravity to the culture tanks. The most important design criteria in sea water based hatcheries are the materials to be used. Materials like plastics, PVC, concrete and wood, which do not corrode in the saline environment, are most commonly used in hatcheries.

Seawater intake

There are numerous designs now in use in the various sea water based hatcheries. These designs are dependent on specific site characteristics, topography, geology, climate etc., Mostly used system of drawing water is through intertidal borewells or through inshore open wells. Low depth intertidal bore wells are suitable in areas where the wave action is minimal. Inshore open wells could be used where the wave action is more in the intertidal zone, and there is no freshwater aquifer in the shoreline.   Fig. 1 - 3 has depicted different water intake systems in hatcheries.

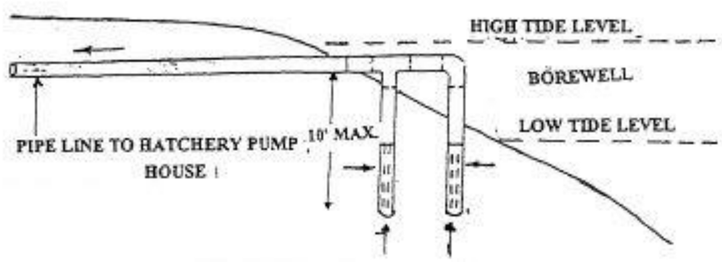

Figure 1 Inter tidal borewell

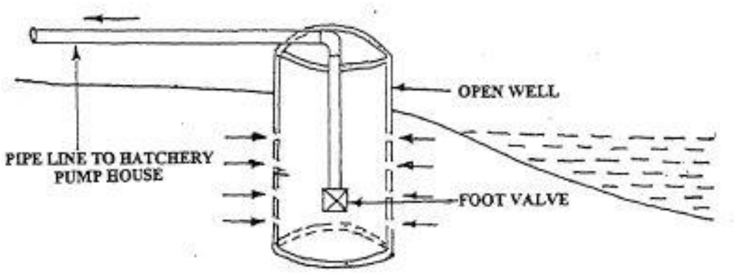

Figure 2 INSHORE BOREWELL

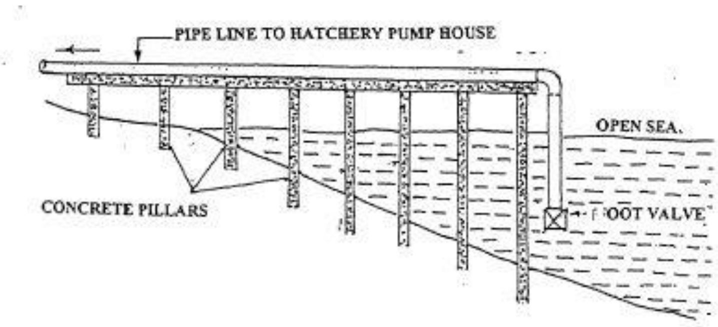

Figure 3 Pumping from open sea

WATER TREATMENT

Hatchery requires clean seawater. If the water is drawn from the open shore, it will contain suspended particles, which are to be removed as a first step before any other treatment. Large suspended particles are easily removed by allowing the water to stand overnight in a settling tank by the process of sedimentation. If the seawater is drawn from intertidal bore wells or inshore wells, then the water will be free from suspended particles, and no sedimentation will be necessary. The next step in the treatment of sea water is the removal of unsettled suspended particles and other living organisms. This could be done by filtration. The filtration is done through a sand-gravel filter, which is simple and most practical. Two types of sand- gravel filters are generally used in the hatcheries -

a) Filtration by gravity and

b) Filtration by pressure.

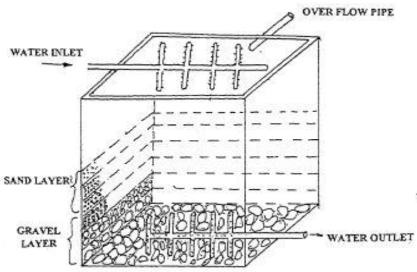

Figure 4 Sand gravel filter by gravity

A simple gravity filter consists of a wooden or concrete tank with layers of gravel and sand. The gravel layer consists of a larger layer at the bottom with medium and smaller layers above it. Similarly, three grades of sand (coarse to fine) is used above the gravel layers. A perforated PVC pipe, embedded at the bottom of the gravel layer and extruding out of the tank, acts as the outlet. The water is pumped into the filter at the top. The water flows through the sand and gravel-bed. The coarse suspended particles are entrapped in the sand bed, and the clean water is taken in through the exit at the tail end.

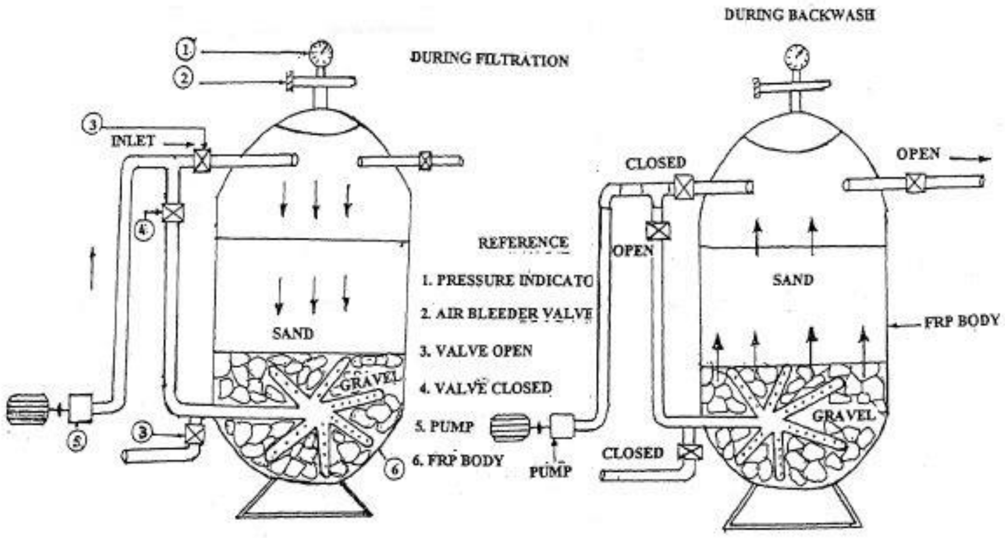

Figure 5 Pressure sand filter

Pressure/ rapid sand filters use the same principle as that of the gravity filters. The difference is that the water passes through the sand and grave1 bed through pressure. The filter housing is made of FRP and is sealed airtight after arranging the sand and gravel in position. The delivery from a pump of required capacity is bound to the filter's inlet, and the filtered water flows through the exit at the same speed as that of the pumped in water. The filtering rate is very high, and therefore, it is called a rapid sand filter. The operation of the filter results in the accumulation of waste materials in the sand bed. The filtration capacity and rate reduce with such accumulation. Cleaning of the sand bed becomes essential. Allowing the water to flow in the return direction, i.e. entering through the gravel and flowing out through sand, is adapted, and provisions for such backwash is given in all the sand filters. Sand filtration removes only coarse materials up to 10 microns in size. Further filtration can be done by using fibre based cartridge filter, which will remove suspended particles of up to 1 micron in size. The fibre filter is enclosed in a non-corrosive housing. The water is pumped through the cartridge to the outlet. It can be easily fitted to the water lines.

Influent water treatment system at CIBA hatchery

In CIBA shrimp hatchery, the seawater is drawn through a bore well sunk in the intertidal zone with the suction point located about 15 meters from the shoreline. It is pumped into a reservoir of 40 t capacity where it is treated with calcium hypochlorite @ 10 ppm. It is then allowed to settle for 24 hours so as the residual chlorine get expelled. The residual chlorine is neutralized with required quantities of sodium thiosulphate and passed through sand filters. The clean seawater passed through cartridge filters and then passed through UV filters before it is used for the hatchery use.

Recirculating Aquaculture System (RAS)

Recirculating Aquaculture System (RAS) can be defined as an aquaculture system that incorporates the treatment and reuse of water with less than 10% of total water volume replaced per day. Water treatment components used in RAS need to accommodate the input of high amounts of feed required to sustain high rates of growth and high stocking densities typically required to meet financial outcomes. Generally, RAS consist of mechanical and biological filtration components, pumps and holding tanks and may include a number of additional water treatment elements that improve water quality and provide disease control within the system.

Components of RAS

Recirculating Aquaculture Systems consist of some typical components which have a certain treatment process.

- Solid waste removal unit
- Biological filtration unit,
- Degassing and oxygenation unit
- Disinfection unit

Waste solids removal

Decomposition of solid wastes and uneaten or indigestible feed produce large quantities of ammonia-nitrogen and consume significant amounts of dissolved oxygen as they decompose (BOD – Biological Oxygen Demand). For this reason, waste solids should be removed from the system as quickly as possible. There are four categories of waste solids: 1) settleable, 2) suspended, and 3) floatable and 4) fine or dissolved solids. Each requires a different RAS component to eliminate or minimize the impact on water quality.

Biofiltration for nitrification

Ammonia and nitrite-nitrogen are byproducts of the metabolism of protein metabolism in feeds (faecal material and decomposition of uneaten feed). If un-ionized ammonia (NH_3), and to a lesser extent nitrite, are allowed to concentrate in the culture system, they will become toxic to the animals in the culture. In RAS, ammonia and nitrite-nitrogen must be removed at the same rate that it is produced in order to maintain a stable culture environment. Biological filtration (biofiltration) is the most commonly used method to control ammonia. It is based on the oxidation of ammonia to nitrite, and finally, the less toxic nitrate, Two groups of bacteria are responsible for this conversion ― *Nitrosomonas* (ammonia) and *Nitrobacter* (nitrite to nitrate). A substrate that has a high specific surface area (large surface area per unit volume) provides an attachment site for the bacteria. Some common substrates include sand or gravel, plastic beads, plastic rings, or plates.

Aeration and degassing

Aeration may be provided by the inherent design of the recirculating system, for example, water discharge through a packed column. However, if the system design does not include aeration, additional equipment such as a blower will be necessary to perform this task.

Carbon dioxide removal

Excess CO_2can be toxic to aquatic species, and therefore biofiltered water must go through a degasification chamber such as a packed column to prevent accumulation.CO_2 is a byproduct of fish/shrimp and bacterial respiration, and it can accumulate within recirculating cyst should be maintained at < 20 ppm to maintain good growing condition. Elevated CO_2 concentrations in water are not toxic when sufficient DO is present.

Disinfection through ozonation and ultraviolet irradiation

An inherent disadvantage of RAS, as opposed to flow-through aquaculture systems, is the threat of disease spreading throughout every tank in the system. The use of chemical or antibiotic treatments can decimate the nitrifying bacteria living within the biofilters and the culture system. An alternative to chemical treatments and a common disease preventative is continuous disinfection of the recycled water using ultraviolet irradiation or ozonation.

Ozone is supplied by an on-site ozone generator (due to its short life span –10 to 20 minutes), and usually through an external contact basin or loop. There, the exposure time can be adjusted to ensure sterilization and any residual ozone is destroyed. Residual ozone entering the culture tanks is highly toxic to crustaceans and fish; ozone in the air also is toxic to humans in low concentrations. Therefore, great care should be taken to vent excess ozone outside the building and generating systems properly installed.

Ultraviolet (UV) radiation can be used to destroy ozone residuals and denature the DNA of microorganisms, causing the microorganisms to die or lose their function. Therefore, the organisms living in water that passes in close proximity to UV will die and the water sterilized. Typically, a UV bulb (similar in design to a fluorescent light bulb) is housed in a quartz cylinder, then placed inside the flow stream pipe (the bulb does not come into direct contact with the water). The efficiency of UV irradiation is determined by: 1) the size of the organism, 2) proximity to the UV source (should be around 0.5 cm), 3) level of penetration of the radiation through the water (influenced by turbidity), 4) exposure time (flow rate relative to the length of the UV tube.

The major advantage of UV treatment is that it is safe and is not harmful to the cultured species, nor does it affect the health of the bacteria within the biofilters, disinfecting water faster than chlorine without cumbersome retention tanks and harmful chemicals and extremely cost effective. The main disadvantages are the requirement for clear water with low suspended solids, the cost of the UV bulbs, and the need for periodic replacement.

Design of RAS system

The reasonable approach for developing an appropriate design for the aquaculture system is based on the mass balance concept. The design for oxygen supply for the cultured animals, design for ammonia removal and calculation of the water requirement need to be arrived at based on the existing condition. The RAS could be designed for maturation, nursery and culture in tank systems based on the requirement. Thus setting up a RAS requires that considerations of costs, RAS unit processes, engineered system integration and engineered equipment selections. Moreover, alternative design solutions for each system and subsystem and component are provided.

RAS for Shrimp maturation

Recirculating systems have been used successfully in fish aquaculture for the past 20 years and are now finding increasing application in shrimp maturation, where production of nauplii increased and improved biosecurity. Properly designed closed systems provide stable water quality, which mimics the environment found at natural oceanic spawning grounds. Fluctuations in temperature, salinity, pH, ammonia, nitrite, and other parameters inhibit shrimp maturation.

In CIBA, RAS for shrimp maturation has been installed, and studies were conducted to ascertain the efficiency of flow through system and recirculation system. and it was clearly proved that the RAS is better for shrimp maturation.

Conclusion

Treatment of water is paramount in a hatchery for the production of healthy shrimp. Though research on water treatments are available site specific treatments needs to be adopted.

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Brood stock development and induced maturation of penaeid shrimp

Shyne Anand, P.S., Balasubramanian, C.P and Vinay, T.N

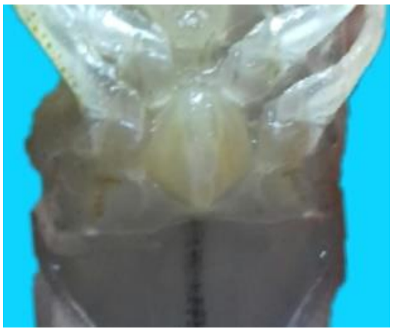
Introduction

Quality seed and feed are the key factors that determine the success of aquaculture. Successful propagation of captive penaeid broodstock relies on shrimp maturation and it's reproductive performance. In general marine penaeid shrimps, adult lives and spawns in the sea. The larvae undergo metamorphosis in the sea, and post larvae migrate to brackishwater environments while juvenile and subadult spend life in coastal estuaries or lagoons. Though reproductive maturity varies with species and habitat, in general, it is reported that in nature male mature at 20g size and female at 28 g size. However, in hatchery broodstock weighs 40-60 g size is generally preferred for seed production.

Penaeid shrimps are grouped into two broad categories based on differences in morphology of the female genital organ or thelycum. In "open thelycum" species mating happens between intermoult male and female, and female retains spermatophore externally for a few hours before spawning. In contrast, in"closed thelycum" species mating happen between a hard male and newly moult female. Female retains the spermatophore until she utilizes the sperm in one or several spawning. The open thelycum females follow a sequence of molt-mature- mate and spawn while closed thelycum species follow molt- mate-mature and spawn pattern. In both groups, males with hard exoskeleton deposit spermatophores into females. Multiple spawns may occur within one intermolt period for both open and closed thelycum species. Most dominant open thelycum species is *Penaeus vannamei* and closed thelycum species are *P.monodon, P.indicus, etc.*

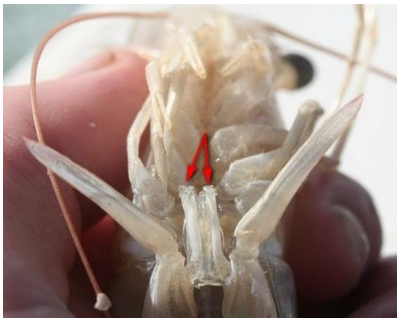
At present global shrimp farming sector dominated by *P. vannamei* credited to fast-growing and disease resistant strains developed through selective breeding programs. In *P.vananmei* mating happens when both male and female are in the hard stage, and after mating, spermatophore can be seen as white sperm plug glued to thelycum. In closed thelycum species such as *P.indicus* or *P.monodon*, the impregnated female can identify as white spermatophore packets inside in the thelycum.

P.indicus male with Petasma



P.indicus with impregnated closed *thelycum*

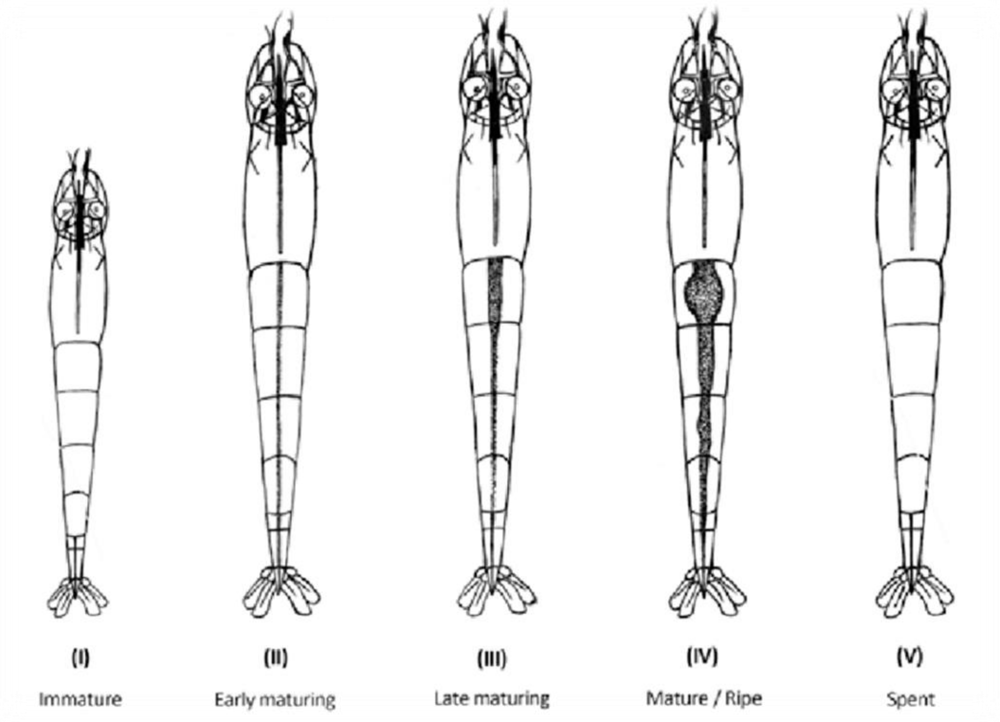
P.monodon closed thelycum

Mature *P.vannamei* female with open thelycum and male with spermatophore (Source: Kannan et al., 2015; Taterka, 2011)

<u>Stages of ovary development</u>: It has five stages of ovary development given below (Fig 1)

1. Stage 1: Immature
2. Stage 2: Early maturing
3. Stage 3: late maturing
4. Stage 4: Gravid/mature
5. Spent



Induced maturation techniques

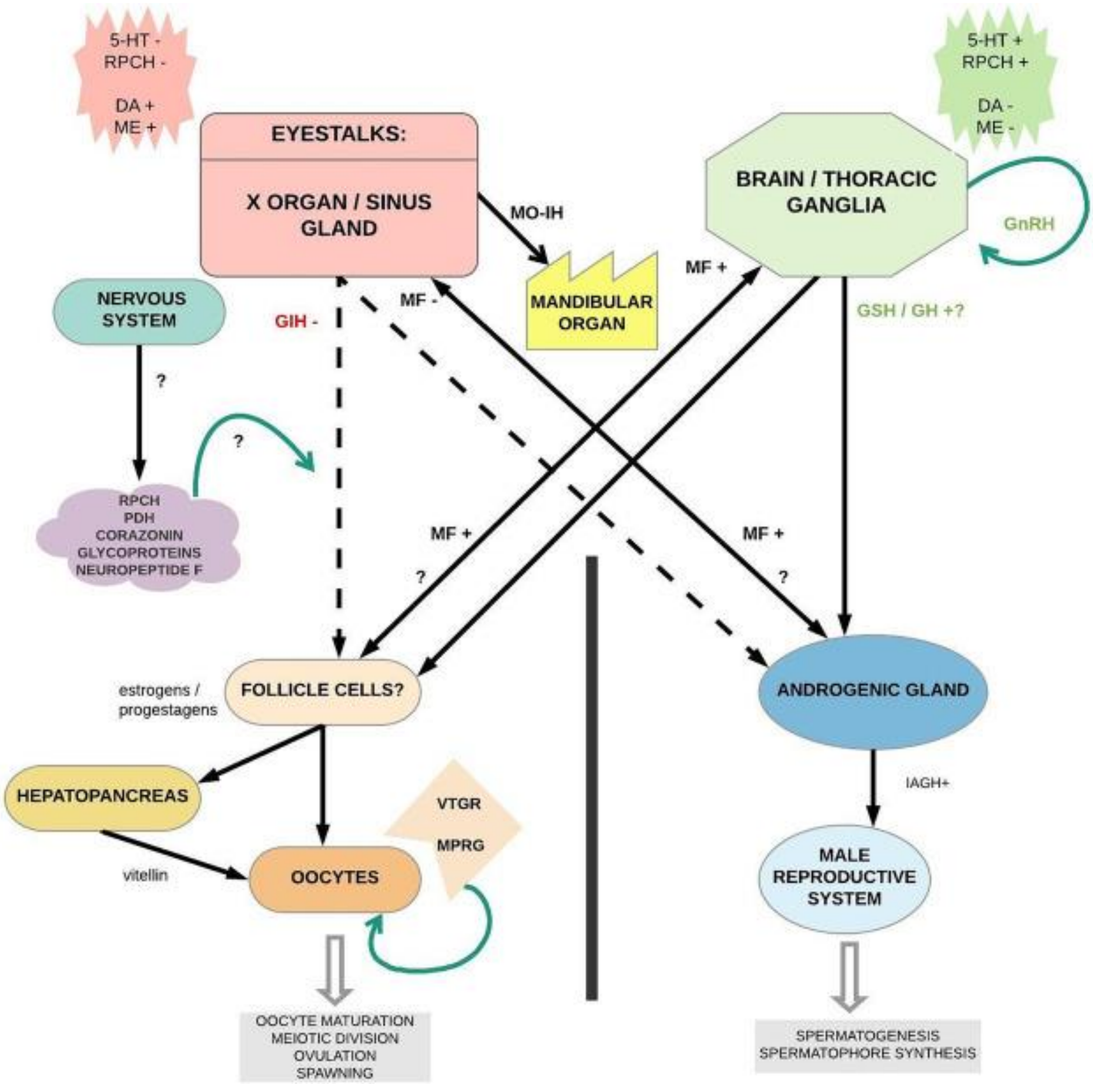
Based on the multifactorial regulation of female shrimp reproduction, a combined molecular therapy need to be applied. The basic model for the shrimp reproductive physiology establishes a cascade and antagonistic hormonal control, based on three levels: (i) central nervous system (CNS)/ x-organ-sinus gland level, (ii) androgenic gland (AG, males)/mandibular organ (MO), and (iii) male/female reproductive system and complementary target tissues (hepatopancreas, appendages) (Nagaraju, 2011). Most known neurohormone belongs to the crustacean hyperglycemic hormone (CHH) family: the vitellogenesis inhibiting hormone (VIH) or gonad-inhibiting hormone (GIH) from the x-organ-sinus gland, negatively regulating oocyte maturation and spermatogenesis.

Eyestalk ablation

Induction of maturation through eyestalk ablation technique is the most commonly and extensively used technique by almost all commercial hatcheries and research facilities worldwide. It is done by removing 2/3rd of the eyestalk with red hot pinchers. The principle behind ESA is X Organ Sinus Gland (XOSG), located in the eyestalks, is the principal neuroendocrine gland in shrimps. This organ produces and stores hormones which regulate various metabolic and physiological activities like vitellogenesis, molting, carbohydrate, protein or lipid metabolism, etc. Removal of one eye stalk reduces gonad-inhibiting hormone level produced by the XO-SG in the body, which in turn stimulate shrimp ovary development. Despite its several advantages, this technique reported to produces poorer quality larvae after successive spawning.

Hormonal manipulation

Alternative techniques to control shrimp reproduction have received little attention, and such studies have mainly concentrated on the injection of various hormones or manipulations of temperature/photoperiod regimes. The role of steroid hormones in crustaceans is reported and noticed that estrogens stimulate vitellogenesis and progesterone induce oocyte maturation. Similarly, intramuscular injection of 17 alpha hydroxyl progesterone, 17 beta-estradiol, etc along with eyestalk ablation found to influence ovarian development and spawning and improve sperm quality in *P. vannamei* and *P.indicus* and *P.monodon*. Endocrine cascade systems such as biogenic amines activate vitellogenesis and indirectly affects meiotic maturation. Neurotransmitters, particularly serotonin, has given the most promising results for inducing ovarian maturation. Serotonin (5-hydroxytryptamine; 5-HT), a neurotransmitter, has shown to induce reproduction. Serotonin is reported to inhibit GIH (gonad-inhibiting hormone), secreted from the X-organ/sinus complex, or stimulate GSH (gonad stimulating hormone) in decapod crustaceans (Tinikul et al., 2008). Serotonin induces ovarian maturation by increasing vitellogenin accumulation in penaeid shrimps. The induction of ovarian maturation and spawning in serotonin/spiperone injected females suggest that the new experimental protocol is a remarkable alternative for the traditional eyestalk ablation. Recently, it has been reported that GnRH plays an important role in the development of ovary in *P. monodon*.



Current knowledge of the endocrine control of penaeid reproduction (Alfaro-Montoya et al., 2019)

Ovarian maturation induction by pheromones

In crustaceans, reproductive pheromones have only been related to mating behavior. The first crustacean sex pheromone was isolated from the crab, *Carcinus maenas* is a nucleotide: uridine diphosphate reported to induces sexual behaviors in males. In penaeid shrimps, the only report suggesting the existence of ovarian maturation inducing pheromones was provided by Alfaro et al. (2004). The authors discovered that by injecting serotonin plus spiperone into cultured *P. vannamei* and wild *P. stylirostris* females, ovarian maturation was activated directly by the combined action of the molecules, but also indirectly in non-treated females.

Ovarian maturation induction by RNA interference

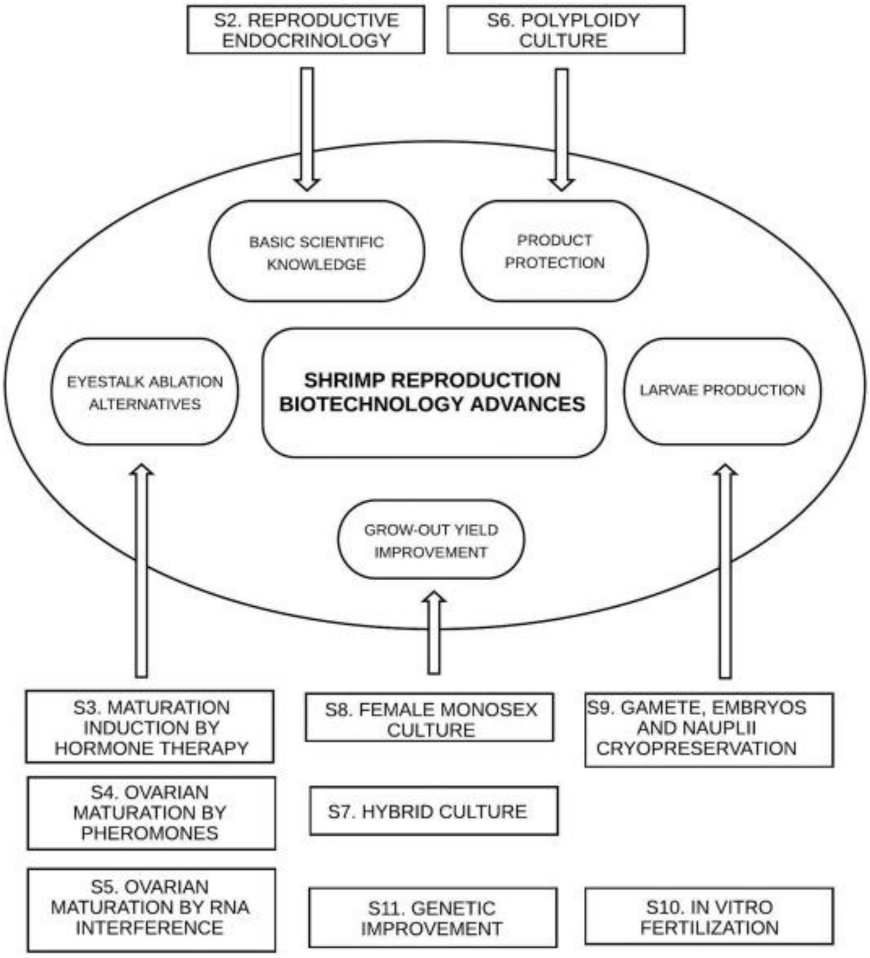
The use of double-stranded RNA (dsRNA) molecules to silence hormonal transcripts by RNA interference (RNAi) has also been considered as an alternative technique to eyestalk ablation of penaeids. Molecular characterization of sinus gland peptides having vitellogenesis-inhibiting activity, the GIH, and the vitellogenin cDNA, was conducted for shrimps demonstrated *in vitro* the influence of GIH on Vg transcripts levels in the ovary using GIH-specific dsRNA. These results highlighted the potential of dsRNA-mediated gene silencing as a powerful tool for more ethical shrimp gonadal maturation.

Polyploidy and Hybrid culture

The induction of polyploidy in penaeid shrimps offers an alternative for improving growth rates, sterility, and genetic protection. A commercially important consequence of this technology is the skewed sex ratio towards females. The culture of hybrid shrimps is another alternative for improving yields in commercial operations based on the principle of hybrid vigor induced by positive heterosis. Hybridization vigor can be achieved intra-specifically, mating individuals of different strains of the same species or inter-specifically, mating individuals of different species.

Environmental manipulation

It is well known that some environmental factors have effects on reproductive performance of penaeid shrimps in hatcheries. In general, photoperiods and temperatures are reported to be necessary for reproduction. The ideal reproductive environment is utmost important for shrimp maturation. Environmental parameters play an important role in maturation. Temperature generally keep at 27-29^{o}C, and male brooder tanks need a comparatively lower temperature to maintain sperm quality, 26-27 ^{o}f for gonad maturation through seawater chillers while female brooders keep at 28-29 ^{o}C. Optimum salinity requirement for maturation is 28-35 ppt and pH 8. A photoperiod of 10-12 h dark and 12-14 h light is reported to be ideal for *P.vannamei* maturation. Photoperiod is maintained at 12D (4 am to 4 pm) and 12 L (4 pm to 4 am) in a day. Ideally female: male ratio is maintained at 1:1 to 1:2 to get maximum hatching. Minimum disturbance or frequent movement must be restricted in the maturation room.



Schematic representation of the ten reproduction-related research subjects identified as potential improving fields for the shrimp industry (Alfaro-Montoya et al., 2019)

Broodstock diet

The quality of maturation diet plays a crucial role in shrimp maturation apart from hormonal manipulation. Hence, it is paramount important to select optimum diet with reliable supply, consistent quality, easy handling, effective in the delivery of immunostimulant, therapeutics or hormones, minimum risk of disease transfer, etc. Fresh feeds are generally considered as ideal for shrimp maturation as they are rich in high levels of polyunsaturated fatty acids, especially arachidonic acid (ARA, 20:4ω6), eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) which are essential for shrimp maturation. At present, the shrimp industry uses a wide variety of fresh feeds like squids, bivalves, polychetes, in combination with other artificial diets. Among these, polychaetes (annelids) and live *Artemia* biomass are considered as indispensable for shrimp maturation due to its better fatty acid profile and the presence of hormonally active substances. Our studies reveal the presence of steroids like hormones, estradiol, and progesterone in the Artemia biomass and its level was comparable to that of live feed polychaete worm.

Similarly, Artemia can be used as a biovehicle for hormone delivery. As many times, fresh feed such as polychaete worms acts as a carrier for viral pathogens. Hence it must be depurated and ozone treated before being fed to the broodstocks. Defreezing the fresh feeds reduces its pathogen load. Broodstock diets must be supplemented with essential vitamins like vitamin E, A, E, Minerals and carotenoid compounds such as astaxanthin and paprika, etc. In commercial hatcheries, broodstock feeding is generally provided at 25% and recently increased up to 50% of body weight at 4 to 6 time per day. A recent study revealed that taurine, a beta-sulfonic amino acid incorporation in shrimp broodstock diet, enhances reproductive and larval cycle performance.

Broodstock maintenance and stocking procedure

Round or rectangular tanks with minimum 5 m dia and 0.5-0.7 m depth having smooth inside is preferable for maturation. Generally flow through based maturation system with 250-300% water is exchanged is followed in shrimp hatcheries though recirculatory aquaculture system is reported to be ideal for shrimp maturation. Average stocking density of brooders are 6-8 number per sq.m, and the stocking density can be increased up to 16 /m^2 if artificial insemination procedure is followed. Generally, it is reported that stocking density must be 0.2-0.3 kg per m2 based on biomass. Segregation of sex and stocking in different tanks are practiced for fed manipulation, sperm quality improvement like maintaining the temperature at 26-27^{0}C temperature, artificial insemination, etc. Artificial insemination is also followed spermatophore from the male is removed by pressing the 5th leg pair by forceps or by electrostimulation (1.5-4v,1.75 A for 2-50 sec), and collected spermatophore is inserted manually in the female receptacle by forceps. Though this process, genetic selection is possible.

Spawning

Fecundity of broodstock of 45-50 g size is reported to yield 1.4-2 lakh egg per spawn. An ablated spawner is reported to spawn about 10-12 times based on the amount of quality maturation diet. Individual spawning is preferred to reduce horizontal transmission of diseases though the majority of the hatcheries follow collective spawning. Every day at 10 pm, female (3/4) stages are collected and stocked in male tanks for mating. Mated females are collected by 4 am (first sourcing) and transfer to spawning tanks. Unmated females, if any transfer to male tanks check for mating (2nd sourcing). Spawning generally starts at 5 to 10 am and spawned females are removed at 10 am and collect the fertilized eggs by 10 am. Depending on the feeding regime, a female batch produces 12 spawn in 3 month period.

Hatching

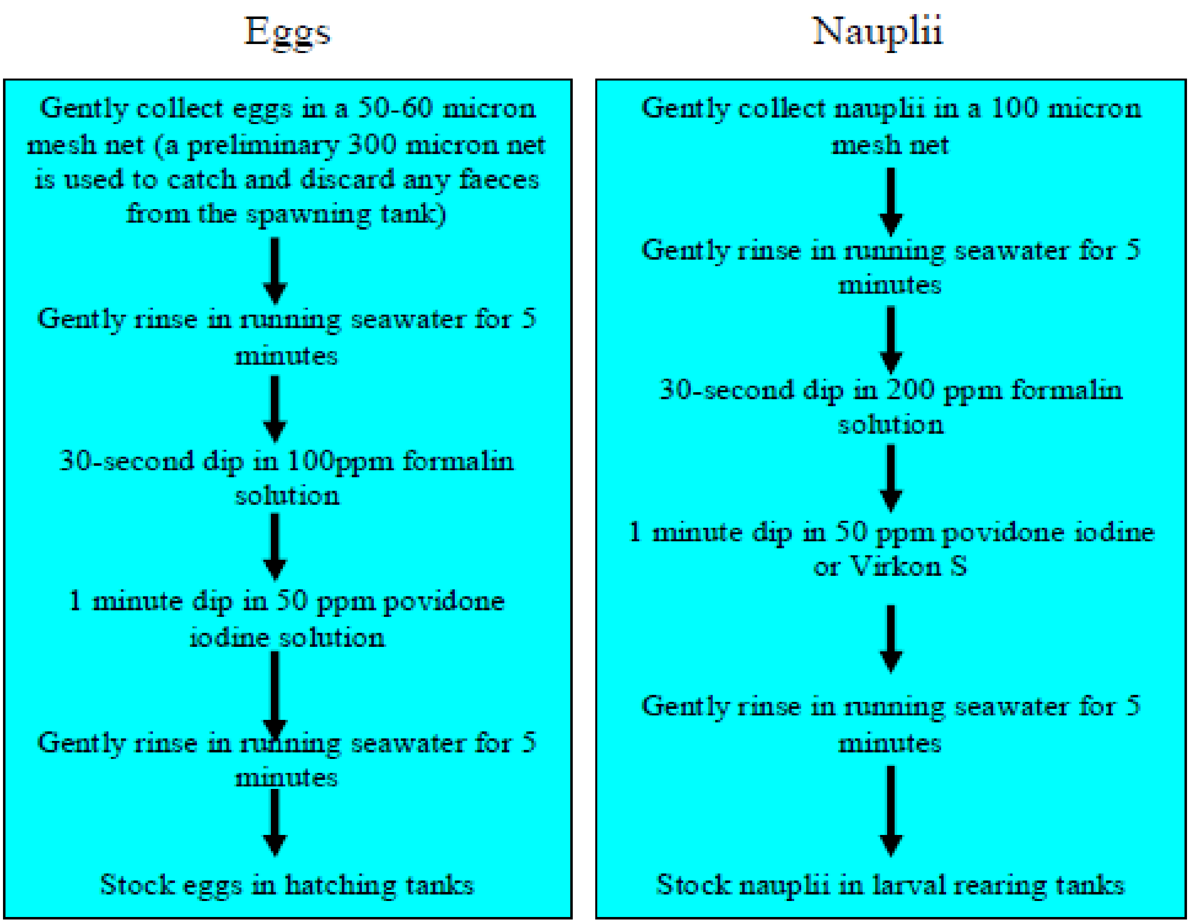
After spawning the spent females were removed from the tanks by a scoop net. The tank water was drained, and the eggs were passed through a 350-micron hand net which retains feces and are collected on a 100 micron net in a harvest bucket. Before transferring the eggs to the hatching tanks, they were washed thoroughly with running sea water at least for 5 minutes and then they were treated with 100 ppm formalin for 30 seconds and 50 ppm iodine for 60 seconds and again washed thoroughly with running sea water for 5 minutes before being placed into hatching tanks (500 L). The number of eggs and the percentage of the fertilized eggs were estimated by using the formula. Eggs stocked in 500L hatching tanks (500L) hatches after 10-12 h. All the hatching tanks are provided with 5ppm EDTA as a chelating agent to reduce the heavy metal load in the water. Periodic shuffling is done to improve hatchability.

$H\% = \frac{Y}{X * 100\%}$ Where H=Hatching rate, Y=Total number of Nauplius and X= Total number of eggs

Selection of nauplii

Positively phototactic larvae are harvested by installing the nauplius harvesting net to the hatching tank's outlet pipe. Fill the harvesting channel with clean sea water until it overflows. Adjust the water level in the harvesting channel to control the strength of water current (from the pipe), so that it is not too strong. Harvested nauplius need to be given dip treatment like formalin (100 ppm) or treflan and can be stocked in larval rearing tanks at 100 number per liter. Hatching percentage above 80% is reported to be good quality, and below 60% is generally discarded.

Washing and disinfection procedure for egg and nauplius



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Larval rearing practices for Penaeid shrimp hatchery

Biju, I.F., Shyne Anand, P.S., Balasubramnian, C.P., Aravind, R and Jose Antony

Introduction

Larval rearing practices in a shrimp hatchery starts with the stocking of the nauplii stage larvae which is the first larval stage after egg hatching. In any penaeid shrimp hatchery, the larval rearing practices are common with slight changes in the management and operating procedures. Maintenance of strict discipline, hygiene practices and biosecurity is necessary for the successful operation of any shrimp hatchery. Good quality filtered and disinfected seawater is must for the larval rearing as well as live feed culture. Monitoring and record keeping until the packing of the seed is required for performance evaluation and preventive maintenance. Water filtration system, aeration equipment and live feed culture are the integral part of the hatchery and should have adequate capacity and backup systems in case of unforeseen situations.

Stocking of larvae

The larval life cycle of shrimp comprises of 12 larval stages before metamorphosing into a post larvae, which resembles a baby shrimp. The shrimp larval rearing practice includes the rearing of the nauplii stage larvae up to the post larvae fifteen stage, where it becomes ready for packing and stocking in the farm pond. The first larval stage of shrimp is known as nauplii stage, which comes out just after hatching of the egg. They are very tiny measuring from 0.30 to 0.58 mm in total length. This larval stage shows positive photo taxis and are attracted towards the light. The nauplius stage comprises of six sub stages and completes it in 1.5 to 2 days. These sub stages differ from each other mainly on the furcal spine formula, which indicates the number of spines on the furca. Identification of these stages can be done by the use of an ordinary lab microscope. Shrimp hatchery procures nauplii stage larvae for stocking from nauplii suppliers or from its own maturation and breeding section with in the hatchery. Nauplii can be transported at a density of 15000 – 30000/litre depending on the time and distance to the hatchery.

The larval rearing is done in 10-12 tonne capacity epoxy coated, ‘V’ shaped cement tanks with bottom drain. These tanks are oval in shape and includes a curved bottom with graduation for monitoring the water level. They are provided with provisions of seawater, freshwater inlet pipes and aeration pipes. The aeration pipe is installed at the bottom of the tank in the form of a perforated pipe and air is provided to this pipe through a feeder pipe from the mainline. The larval rearing tanks are washed thoroughly with bleach/commercial acid and further with liquid detergent. These tanks are allowed to dry and after which filtered seawater is pumped into the them prior to stocking of the larvae. Titanium heaters are also provided in the tank during cooler seasons to maintain the temperature at 28°C. As the first step of stocking the larval rearing tanks are filled to the 3-4 tonne mark and aerated. Upon receiving the larvae, the total count is estimated and a prophylactic treatment to remove fungus and other parasites is given prior to stocking in the tanks. Estimating the total count is necessary, as we have to maintain a required stocking density in the tanks.

Estimation of the total count and the prophylactic treatment can be done as follows:

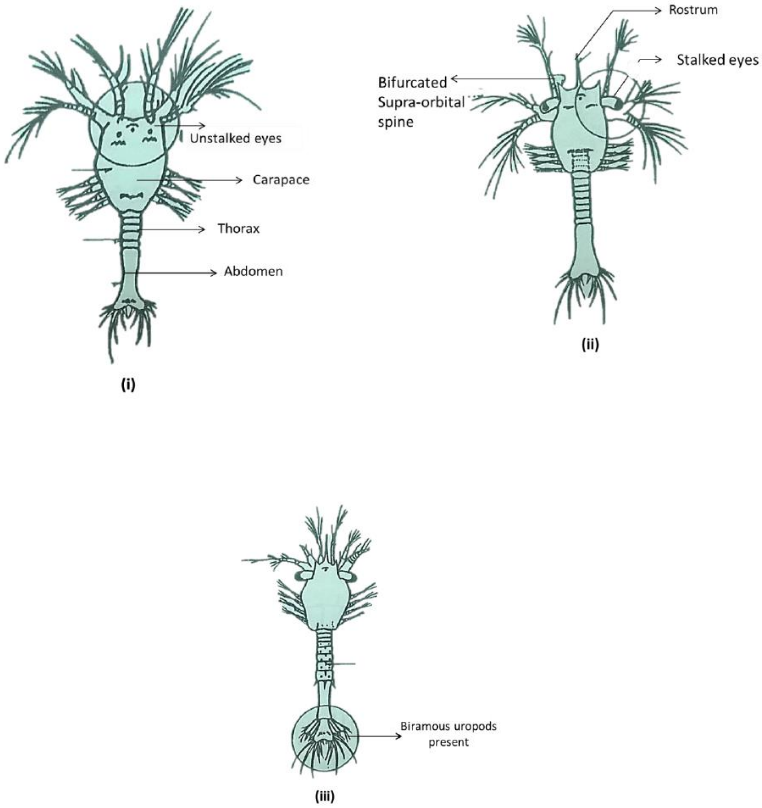
- The nauplii count is estimated by using the volumetric method
- The nauplii is concentrated in a graduated can of 100 litre capacity and a sample of 10 to 50 ml is taken using a beaker.
- The number of larvae in the sample is counted manually and the same is repeated three times. The average of this figure is taken and the total number of larvae present in the can is estimated.
- Prophylactic treatment is given by dipping the larvae in formalin and treflan solutions.
- Prepare the formalin – 200ppm and iodophore – 100ppm solution in a 10litre tub and filtered seawater in another two tubs.
- Collect the larvae to be disinfected in a hand net (50micron), dip in formalin solution for 30 seconds, and thoroughly rinse in the filtered seawater.
- Repeat the step for iodophore and rinse in clean seawater and hold in a container until stocking. The stocking density in the holding container should be 20000 – 40000/litre with continuous aeration.

Once the counting and disinfection is over the larvae is ready to be stocked in the larval rearing tanks. Around 1million to 1.2million nauplii is stocked in to a larval rearing tank of 10-12tonne capacity. A known volume from the Nauplius holding tank is taken and slowly added to the LRT such that the tank contains around 1million nauplii. Higher stocking density is maintained in the initial days so that, while the larvae becomes post larvae the tank will have a stocking density of 50-100PL/litre. Mild aeration is given and the tanks are covered with black sheets to prevent strong light. Nauplii stage is a non-feeding stage, as they will be utilizing the yolk reserve in the body. Periodically sample is taken and observed under the microscope for stage changes. After 36-40 hrs, they transform into Protozoea stage, where they starts feeding on microalgae. Fresh micro algae feed is given the moment larvae transform into the Protozoea stage.

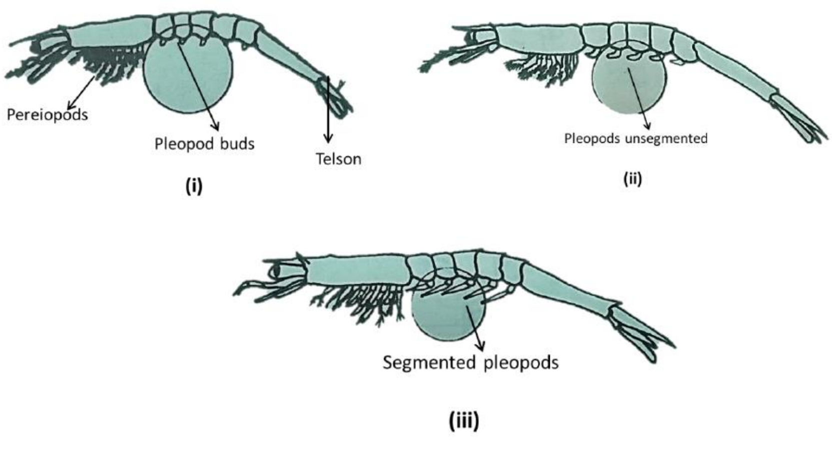
Protozoea larvae and feed management

As the larvae transforms into the protozoea stage feeding commences. Fresh, good quality culture of micro algae is normally pumped into the larval rearing tanks for feeding the larvae. Every shrimp hatchery will essentially have an indoor micro algae lab and outdoor mass culture facility. The indoor algal lab maintains the pure stock of the desired micro algae for culture and the outdoor mass culture facility mass-produces the algal culture for feeding the larvae. Micro algal lab maintenance and feeding management should be done with most care because these are two crucial steps, which decides the survival of the larvae. The sought after micro algae species for shrimp hatchery are the diatoms. *Chaetoceros* spp., *Thalassiosira* spp, *Skeletonema* spp. are the most common diatoms used in the shrimp hatchery due to their ease of culturing and maintenance. Other species like *Tetraselmis* are also used but not so common.

Identification of the protozoea stages of shrimp larvae



Identification of the Mysis stages of shrimp larvae



The protozoea stage can be easily identified from the nauplii stage by observing under the microsocope. The protozoea stage comprises of three sub stages and each stage takes around 30-48hrs to complete the cycle. While feeding the larvae, maintaining the correct density of micro algal cells in the rearing tank is very important. For the protozoea stages, minimum of 1lakh cells/ml has to be maintained in the larval rearing tanks. To properly maintain this density in the rearing tank, the density of algae in the outdoor mass culture tank should be calculated. The density of algae is normally estimated by the use of the counting chamber

(Haemocytometer) mounted on a microscope. Once the cell density of the mass culture of algae is known, the amount to be pumped into the tank is calculated and directly pumped to the tanks. In commercial shrimp hatcheries around 250-300litres of algal culture is pumped both morning and evening. Monitoring of the larval stage is done by periodically collecting samples from the tank for observation and while sampling note the swimming behaviour and presence of faecal threads. Actively feeding zoea larva will be swimming freely and will not settle to the bottom. The presence of faecal threads in the body is an indication of normal feeding.

During the zoea stages, water exchange is not performed and to maintain water quality fresh seawater is pumped into the tank if required after monitoring the tank health. As the algae and fresh seawater is added on daily basis, the water level in the tank will be increasing day by day and will reduce the stocking density of the larvae. Apart from the micro algal feed, other artificial diets are also given to the larvae. One such feed is the micro coated or micro encapsulated feeds. These feeds are available in different forms and particle sizes for providing to different larval stages. Feeds of particle size 50-60 micron is used during the zoea stages and is given at the rate of 20gm for 1million larvae/day. If the larvae is healthy and developing properly within 4-6 days the zoea larvae transforms into Mysis stage.

Rearing of Mysis stage larvae

When the larvae metamorphose into Mysis stage, behavioural changes are observed in the swimming behaviour. The larvae starts to swim upside down in the Mysis stage and jerking movement is observed. The Mysis stage also comprises of three sub stages and each stage takes 1-2 days to complete. In the Mysis stage micro algae is provided as major feed and the same procedure is followed as in the zoea stage. Artificial feeds of size 100 micron is used in the Mysis stage and is given at the rate of 20gm for 1million larvae/day. Fresh seawater is added into the tank during these days to make up the volume and to maintain the water quality. In the final stages of the Mysis phase, water exchange is performed in the tanks by using a siphoning conduit covered with 200micron net to retain the animals in the tank.

Post larval rearing

The Mysis stage transforms into post larvae and resembles like a baby shrimp. The post larvae in the initial stages swims in the column and in the later stages prefers the bottom layer of the tank. Feeding with microalgae is continued in the post larval stage although the cell density is reduced. The major feed provided to the post larvae is live feed artemia. Artemia also known as brine shrimp is a tiny crustacean mainly seen in the slat pans. The hatched out larvae of this organism i.e is known as the artemia nauplii and is rich in yolk content with essential nutrients for growth of the larvae. Artemia is an inevitable part of any hatchery, be it shrimp, fish or ornamental fish hatchery due to its availability and ease in use as a live feed. Artemia is procured in the form of cysts, which can be hatched and the freshly hatched out larvae can be given to the post larvae as feed. Now a days hatched out readymade artemia nauplii is available in the market, which can be directly fed to the larvae, thus saving time and labour for the preparation of the live feed. Once the larvae becomes the post larvae, the days are counted and each day the post larvae is considered one day old. On this context, the post larvae of five days old will be called as PL5. On all these days artemia is given as feed in both morning and evening. Algal culture is also pumped into the rearing tank, but a lower cell density is maintained. Along with the artemia other artificial feeds of size 200-300micron is also given to the post larvae. Once the post larvae reaches PL5 stage they are transferred into bigger nursery tanks normally called as post larval rearing tanks.

The post larval rearing tanks are flat bottom tanks and are bigger in size with capacity ranging from 10-20 tonnes. In the PLRT the larvae is reared up to PL15-18 and up to the time of packing. In some hatcheries, the post larval rearing is also completed in the LRT. As the post larvae grows artemia feeding is reduced and the artificial feeds are increased. Water exchange is also done in the tanks at the rate of 50%/day and addition of freshwater is done to reduce the salinity to promote the post larval growth.

Packing and transportation

While packing the post larvae of adequate size and quality is concentrated in a conditioning tank with aeration. Before transferring the larvae into the conditioning tank the salinity level is adjusted if the customer has requested for a particular salinity. The temperature of the conditioning tank is reduced gradually to 25-26°C to acclimatize the larvae to lower temperature. Lower temperature reduces the metabolic activity of the animal and reduces stress during transport. Packing is done in plastic bags filled with five litres of water and 1500-2500 larvae depending upon the distance and time of transportation. The larvae is transferred into bags using scoops of various sizes. In order to estimate the number of larvae a trial scoop is done and the larvae is counted, accordingly the size of the scoop is adjusted to get the desired number of larvae/scoop. Finally, the bag is packed with oxygen filling and tied with rubber band. These bags are packed inside the polystyrene box for transport by air/road.

Broodstock management and Larviculture of mud crab

Balasubramanian, C.P, Shyne Anand, P.S., Biju, I.F., Aravind, R and Sivagnanam

Aquaculture of mud crabs (*Scylla* spp) dates back to 1890 in Guangdong, China (Shan and Lain 1994). Since 1970s, steady interest has been shown in culture of *Scylla* spp in many tropical Asian countries. Farming of mud crab is considered to be an important and valuable industry, offering advantages from a number of aspects: 1) uncomplicated technology, 2) abandoned shrimp ponds can be converted, 3) international markets, 4) native species to many tropical Asian countries, 5) easy transportation, potential for rural as well as industrialized aquaculture, 6) individual animals are valued in contrast to penaeid shrimps and 7) resilience of resources. However, crab aquaculture is severely constrained by the unperfected hatchery technology. This article summarizes various aspects of mud crab biology and hatchery technology.

Biology of Mud crabs

Taxonomy

Taxonomy of genus *Scylla* has been considerably confused. Estampador (1949) recognized three species and one variety. His classification was mainly based on coloration, morphological characters and behavior. Although many authors accepted Estampodaor's classification, Stephenson and Campel (1960) concluded that there was insufficient evidence for separation of species beyond mono-specific term *Scylla serrata*. Recently, the taxonomy of genus *Scylla* revised and confirmed the existence of four species (Table 1; Figure 1) based on morphometric analysis, allozyme electrophoresis and mitochondrial sequences. However, insufficient evidences from Indian mud crab species (Kathirvel, Personnel communication) to support the classification suggested by Keenan et al (1998), in this article, the classification suggested by Kathirvel and Sreenivasagam (1991) is used. The following Table provides the status of nomenclature of mud crab species

Table 1: Taxonomic status of mud crabs *Scylla* spp

Kathirvel and Srinivasagam (1992)	Estampador (1949)	Keenan et at (1998)
Scylla tranquebarica	*Scylla oceanica*	*Scylla serrata*
	Scylla tranquebarica	*Scylla tranquebarica*
Scylla serrata	*Scylla serrate*	*Scylla olivacea*
Not reported	*Scylla serrata var. paramamosain*	*Scylla paramamosain*

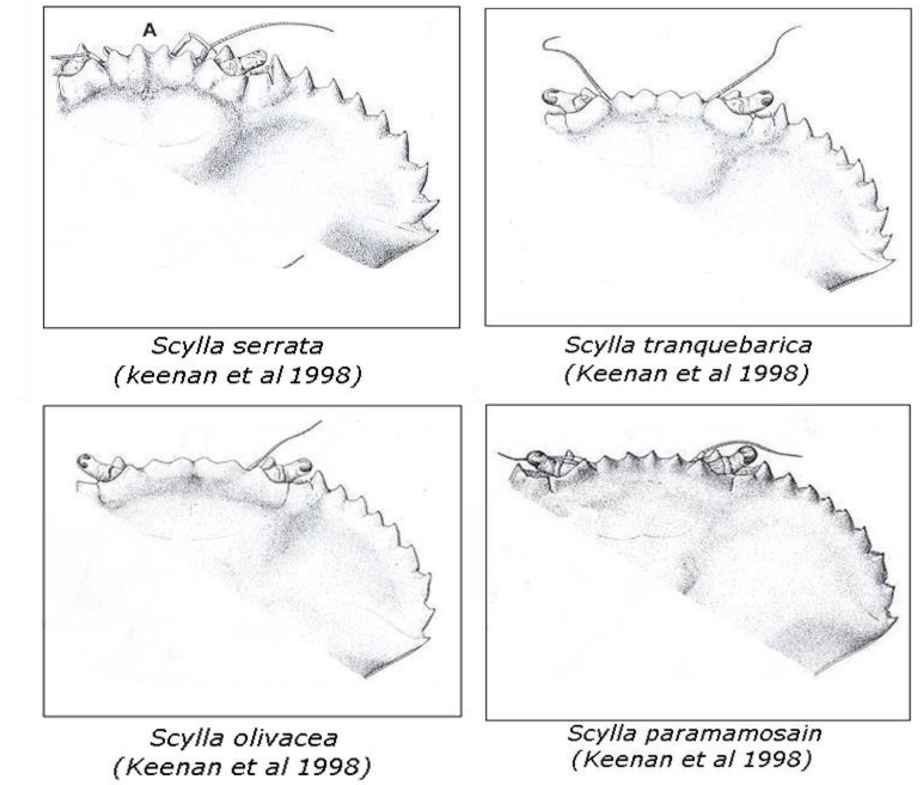


Figure 1 Taxonomic features of different mud crab species (After Keenan et al 1998)

Life History

Mud crab’s natural history can be considered as catadromous: adult spawn in the open ocean but young migrate inshore. The various stages of development are shown in Fig 2.

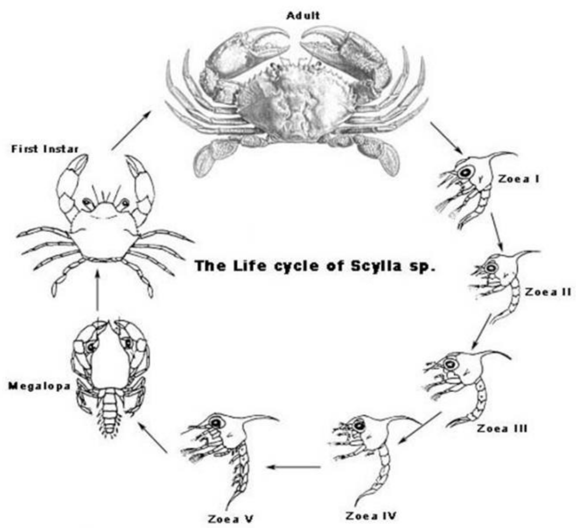


Figure 2 Generalized life cycle of *Scylla* spp.

Biology of Crab Reproduction

The sex of crabs can easily be determined by external features. Male crabs are characterized by inverted 'T' Shaped abdomen, whereas in females the abdomen is semicircular. In addition the male has relatively larger chelae, and a general trimness for body contour than females. Males have two pleopods that modified as copulatory organs on the first and second abdominal segments. In the case of females first four abdominal segments carry pleopods, which are biramous and possess setae for attachments of eggs for brooding.

The female reproductive system comprises a pair of ovaries, a pair of spermatheca (= seminal receptacle) and a pair of vagina. The ovary is 'H' shaped and located dorsally just beneath the carapace. The horns of ovary extends anterolaterally from either side of the gastric mill and dorsal to the hepatopancreas. Two posterior horns, which lie ventral to the heart, extend posteriorly on either side of the intestine on either side of the intestine. The seminal receptacle arises from mid lateral border of the posterior horns. Each antennal receptacle leads into a narrow vaginal tube which further open outside through small circular gonopore situated ventrally. Eggs are produced in the paired ovaries. Sperms produced in the testes opens into coiled tubes (vas deferens) that package mature sperms into gelatinous bundles (spermatophore) for transfer to females. In natural conditions mud crabs attain sexual maturity at between 18 and 24 months.

Mating takes place in the estuarine environment, after which female crabs migrate to the sea where spawning takes place (Arriola 1940, Ong 1966). Berried *S. serrata* females have been caught in trawl nets up to 80 km from the shore in Australia (Poovichiranon 1992, Hill 1994). Spawning appears to occur throughout the year with some seasonal peaks (Heasman et al 1985, Quinn and Kojis 1987). These peaks seem to be related to seasonal rainfall for tropical populations, while in temperate regions reproduction is more strongly related to temperature, with a peak in spawning activity in the summer months (Heasman *et al.* 1985). *S. serrata* is highly fecund with up to 8.36 million eggs per female (Mann *et al.* 1999). The zoeal larvae develop and remain in the open ocean until they reach the megalopa stage, after which they migrate back into the estuarine environment (Keenan 1999). Little is known about the oceanic phases of the life cycle.

Mating

The mature female releases a chemical attractant (pheromone) in to the water, which attract males. The successful male picks up the female and carries her around for several days until she molts. Copulation can occur only when females are in soft shell condition. Male deposits Spermatophore inside the female storage sac (spermatheca) by using male first pleopods. The Spermatophore can remain viable, until fertilization takes place, for weeks or even months. When the eggs complete vitellogenesis they are passed down and fertilized by stored sperms, and extruded onto pleopods. The eggs adhere to the pleopod hairs and female is said to be berry or ovigerous.

Ovarian development

The classification of ovarian maturation provides a guide for broodstock management in hatchery facilities. For hatchery operation, animals with ripe or late maturing ovaries should be selected to minimize the use of resources such as time and money. The colour, size and texture of ovary of mud crabs are closely related to its cellular development. Based on external morphology and light microscopy, Quinitio et al 2007 classified the ovarian development stages of *S. serrata* into five (Table 2)

Table 2: Characteristics of ovarian stages of Scylla tranquebarica (=S. serrata); adopted from Quinitio et al 2007

Ovarian stage	External description	Histological description
Immature	Ovary thread-like; sometimes difficult to recognize from other tissues; transparent to translucent	Oogonia, oocytes and follicle cells apparent in the ovarian lobe; follicle cells found around the periphery of the lobes and an area among groups of oogonia and oocytes; oogonial nuclei in different stages of mitosis and meiosis Thin ovary; translucent to off white Lobes clearly separated by connective tissues; follicle cells with variable shapes gradually enclose the oocytes; more advance oocytes found in the periphery
Early maturing	Ovary increases in size; yellow	2 Early maturing Oocyte diameter increases in size; small yolk globules start to appear in bigger oocytes; follicle cells around the oocytes (Fig. 2)
Late maturing	Massive increase in ovarian size; lobules apparent; light orange	Yolk globules occur in the cytoplasm with larger globular inclusions toward periphery; follicle cells hardly recognizable; a few small oocytes visible
Fully mature	Lobules swollen with large ova; ovary occupies available space in body cavity; orange to dark orange	Large yolk globules in the entire cytoplasm; nucleus small; follicle cells hardly see
Spent	Ovary similar to Stage 2 or smaller than Stage 3;	Yellow to light orange and sometimes with dark orange on some parts of ovary; flaccid Oocytes of various stages present; yolky oocytes not expelled still recognizable; atretic oocytes evident

Incubation

Mud crabs brood their eggs, as all other pleocemata. During the incubation period, females stop feeding and therefore animals generally avoid ‘baited lift nets and ‘traps’. Egg incubation period generally varies from 7 to 14 days, but the duration of incubation is greatly influenced by the rearing water temperature. Egg incubation period is tested at different temperature (20 to 30 ºC) and found that incubation period decreased exponentially with increasing temperature.

Larval stages

There are five zoeal stages passing through five molts to reach the megalopa stage. At a salinity of 31 ppt development from zoea 1 to megalopa requires 16-18 days; each zoeal stage takes minimum period of 3-4 days before it molts in to the next stage. The megalopa takes 11-12 days before it molts into the first crab stage; at lower salinity in the range of 21-27 ppt this period is reduced to 7-8 days. The faster rate of megalopa in lower salinity indicates that the megalopa in nature move shoreward into brackish water.

Description of Zoea (From Ong 1966)

The zoea are of typical brachyuran type with long rostral and dorsal spines. The abdomen in all stages have has lateral knobs on second and third pleomeres. Identifying characteristics of different zoeal stage of S. tranquebarica is given tin the Table 3

First zoea: Body length 1.15 mm; eyes sessile. Antenna unsegmented and bears short setae apically. Mandible is broad with two large teeth and serrated edges. Maxillule with two segmented endopodite; maxilla with un-segmented endopodite; the first and second maxillipeds bears four natatory setae. The abdomen is made up of five pleomeres. The telson bears a pair of long dorsolateral spines.

Second Zoea: Body length 1.51 mm; eyes stocked; Exopodite of both maxilliped bear six natatory setae. Telson has a pair of small setae at the inner margin of furca.

Table 3: Summary of different zoeal characteristics of *Scylla tranquebarica* (Ong 1967)

Stages	**Size (mm)**	**Eyes**	**Setae (2 nd maxilliped)**	**Appendages (thoracic)**	**Setae (middle furca)**
Zoea 1	1.1	Sessile	4	Nil	3 pairs
Zoea 2	1.5	Stalked	6	Nil	4 pairs
Zoea 3	1.9	Stalked	9	Starts developing	4 pairs
Zoea 4	2.4	Stalked	10	Large	4 pairs+middle one
Zoea 5	3.4	Stalked	12	Large	5 pairs

Third Zoea: Body length 1.9; Larger antennule than second zoea; antenna has developed a small bud. Exopodite of second maxilliped with 9 setae.

Fourth Zoea: Body length 2.4 mm; Antennule bears aesthets in a terminal group and a subterminal group; Flagellum of antenna elongated; first maxilliped bears 10 natatory setae; second maxilliped bears 10 natatory setae and one or two short setae. Rudiments of third maxilliped appears.Abdomen has bud on pleormeres 2-6. The telson grows additional setae between the innermost pair.

Fifth Zoea: Body length 3.43 mm; first maxilliped bears 11 long setae; second maxilliped has 12 setae. All the pereiopods are elongated and shows the signs of segmentation. Pleopod buds are well developed. Five pairs of setae on the telson furca.

Megalopa: Single megalopa stage similar to other portunids; carapace length 2.18 mm; carapace width 1.52 abdominal length 1.87. The abdomen has five pair of pleopods.

Hatchery production of mud crabs

This section is dealt with three subsections: Facility, Broodstock management and Larval rearing. For the development of mud crab hatchery, most of the shrimp hatchery can be converted into crab hatchery.

Facilities

Broodstock and larval rearing tanks: Tanks may be made of concrete, fibreglas, or wood lined with rubberized canvas. These can be either circular, oval, or rectangular. However, rounded corners are preferable due to more effective water circulation. Tank capacity may vary from 1-10 mt for broodstock and 1-5 mt for larval rearing tanks.

Algal culture tanks: The green phytoplankton, *Chlorella* is needed for rotifer, *Brachionus*

Algal tanks must be shallow to allow enough light penetration. Barchionus are cultured in 5-10 mt tanks.

Spawning tanks: It is advantageous to have smaller round tanks with volumes ranging from 300 to 500 L tanks where berried tanks are held and allowed to hatch their eggs.

Artemia hatching tanks: Nauplii of Artemia or brine shrimp is protein rich organisms give to larvae starting second or third zoea. Tank capacity of Artemia varies from 30 to 50 L.

Reservoir: Storage tanks are necessary for chlorination and holding of filtered and treated water for daily use. An elevated storage tank that can distribute seawater to other tanks by gravity is advantages.

Seawater system: Seawater may be pumped from the sea or sump pit. Water is passed through sand filter, which is usually elevated prior to storage.

Other equipments and accessories: Other equipments and accessory such as refrigerator, weighing balances, refractometer, pH meter and drainers, etc. are equally important in hatchery operations.

Broodstock management

Females of *S. tranquebarica* can be obtained form fishers or landing centres. Male crabs are not required for hatchery operations as almost all matured crabs in wild would have mated (Ezilarassy and Subramoniam,). Animals range in size above 500 g (S. tranquebarica) and above 300 g (S. *serrata*) should be selected for larval production. Further, females were identified as being matured by their wide, dark, U- shaped abdomen fringed with setae. Immature females were typically characterized by having an abdomen resembling that of male with slightly convex side and without setae. Maturity can be assessed by observing through gap at the junction of carapace and abdomen (Fig.3)

Fig. 3 In vivo evaluation of ovarian stage of *Scylla tanquebarica*

Transport: Crabs for transport are tied with twine to render the claws immobile. They can be kept out of water in cardboard cartons for two days. The bottom and sides of containers are lined with damp mangrove leaves, wooden shavings, or damp sackings. As dehydration affects survival of crabs, it should not be subjected to drying winds during transport. Likewise exposure of direct sunlight for long period could lower survival.

Disinfection: Under culture conditions, the ability to control disease is vital because the potential for pathogen proliferation increases with the density of cultured animals. Formalin has extensively been used in crustacean culture for disinfecting and disease prevention. Therefore, newly caught animals should be disinfected with formalin to reduce the number of symbionts and parasites. Formalin doses and exposure time varies widely between studies with doses ranging from 100 ppm for 1 h to 50 ppm for 20 min. (see the box 1 for calculation of formalin)

Eyestalk ablation: As the occurrence of berried crabs in nature is rare, it is essential to develop ovigerous crabs in captivity. Eyestalks are the sites of gonad inhibiting hormones and therefore the removal of eyestalks accelerate the gonad development and spawning. One of the eyestalk is removed. Intact animals can also be used as broodstock, however, the time to get ovigerous crab extended according to the ovarian stages of the animal. Reproductive performance of intact animals are significantly greater in intact animals (Millanema and Quiniito 2000. Table 4)

A sandy substratum should be provided in the spawning tank. Female crabs kept in a tank that has bare floor may often drop their eggs during spawning because eggs fail to remain securely attached to their pleopods. Half of the broodstock tank can be provided with 10 cm sand layer and another half can be bare floor for feeding purpose (Fig 3). Alternatively sand filled trays can be provided (Fig 4). Crabs can be stocked at the rate of one animal per 1 mt or one per sq.m

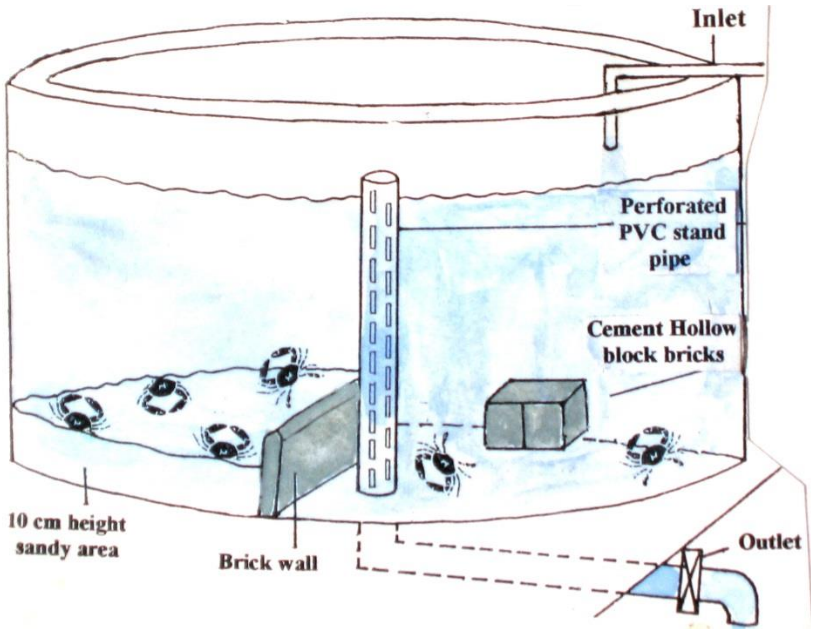


Fig 3 Broodstock tank for *Scylla* spp. Half of the portion is provided with a sandy substratum

Fig 4: Broodstock tank of *Scylla* spp sand filled basin are provided for spawning (after Churchil,2003)

Box 1

To calculate how much formalin should be added to provide a concentration of 100 ppm

Formula:

$$\mathbf{C_1V_1 = C_2V_2}$$

C_1 = concentration of the formalin in the bottle

C_2 = concentration of formalin needed in the tank (100 ppm)

V_1 = Volume of the formalin needed from the bottle

V_2 = Volume of water in tank

Treating larvae with formalin

The prawn larvae in LRTs should be treated every second day with 30 ppm formalin for up to 1 hour, to reduce the incidence of fungus and protozoan infestations.

Formalin may be sold almost pure (100%) or as a 40% solution. Read the label on the bottle to determine the concentration.

100% formalin represents 1,000,000 ppm

40% represents 400,000 ppm.

To calculate how much formalin to add to an LRT to provide a concentration of 30 ppm, use the formula:

$C_1 V_1 = C_2 V_2$

where: C_1 is the concentration of formalin in the bottle

Table 4 Reproductive performance of ablated and intact Scylla serrata (=S.tranquebarica) (Millanema and Quintio 2000)

Table 4 .Reproductive performance of ablated and intact Scylla serrata (=S. tranquebarica) (Millamena and Quinitio 2000)

Variables	Ablated	Intact
No viable spawning	8(40%)	12(60%)
Mean eggs per BW	4437	5124
Mean eggs fertilization rate (%)	58	80
Total No of Zoea (million)	15.67	20.49
Broodstock survival (%)	42	83

Feeds and Feeding: Broodstocks are fed with natural feeds such as molluscs, polychaete at the rate of 10% body weight. Un eaten feeds should be removed daily by siphoning the tank prior cleaning

Water management: Seawater with 28-35 ppt is used for crab broodstock, and water in the tank is changed daily. Sand substrate should be cleaned twice week.

Spawning and hatching: Crabs should be checked daily to know whether spawning has occurred or not. Once crabs spawned they are placed in the basin containing 150 ppm formalin for 30 min., and stocked individually in 300-500 L spawning tanks for subsequent spawning.

Larval rearing operations

Preparation of larval tanks for stocking: The tanks should be disinfected with 200 ppm chlorine water for 8-10 h, and scrubbed with a mixture of 200 ppm Chlorine and 5% detergent by using sponge pads. Then the tanks are thoroughly rinsed with fresh water and drained at least for 24 h. Just before filling the tanks it should be rinsed with fresh water. Before stocking zoea, algae (*Chlorella*) should be added at the rate of 50, 000 cells/ml. The tank water should be aerated mildly. Micro algae do not provide any nutritional benefits; it may enhance the water quality.

Acclimation and stocking: The larvae are estimated in the spawning tank directly. Aeration should be taken out before collecting the larvae, and the waste products settled down at the bottom should be siphoned out. Only active larvae are stoked in the larval rearing tank at 10 – 50 individual per litre. Active larvae are photo tactic; hence they swim up to the surface. The zoea received from the hatching tanks should be acclimatized by adding the larval tank water to the acclimatization basin. The acclimatized zoea can be released slowly in to the tank in small quantities.

Feeding: The most critical component of the mass larval rearing of aqua cultured species is the standardization of feeding regime. The feeding regime for mass rearing of *Scylla* has yet to be standardized. Nutrition has been suggested as a possible cause of mass mortalities experienced during the mud crab larval rearing. Absence of an optimal feeding regime may be the fore most reason for the failure of hatchery production of mud crab larvae. Many experiments were conducted in India and elsewhere using a variety of live feed organisms such as veliger of oysters, copepods, rotifers, Artemia nauplii and micro algae. Trials were conducted using these live feed organisms individually or in combination. Heasman and Fielder (1983) reported their highest survival of 26% form zoea to first crab instar when larvae fed solely on *Artemia* nauplii whereas Marichamy and Rajapakyam (1991) reported a maximum survival from first zoea to first crab instar when they used a combination of rotifer and *Artemia* nauplii. In India Anil and Suseelan (1999) conducted experiments on feeding of *S. tranquebarica* (as *S. oceanica*). They used 3 feed combinations: 1) frozen *Artemia* nauplii, rotifer and micro encapsulated feed 2) Frozen *Artemia* nauplii, rotifer and *Chlorella* and 3) *Artemia* nauplii in suspension in addition to the fresh *Artemia* nauplii (15 -20 individual/ ml), rotifer (Brachionus 20 individual/ ml) with antibacterial compound prefuran. The best survival obtained for the third combination (23%). Although *Artemia* alone can be used and successful larval production is achieved by some authors, most of the authors reported with convincing evidence that rotifer is an indispensable component of mud crab larval rearing. Rotifers are significantly smaller (0.5 µg and 45 -200 µm) than Artemia nauplii (2.7 µg and 428 -517 µm) and less vigorous as well. Measurements of feeding appendages of *S. serrata* larval stages suggest that the optimum food size for Z1 larvae ranges from 100 to 200 µm. Further, early post larvae show a clear preference to slow moving rotifers. There fore Z1 to Z2 should be provided with rotifer. Qunintio et al 2001 suggested that rotifer, should be fed through out the larval rearing cycle. The maintenance of rotifer culture for a long period requires enormous resources, therefore, use of rotifer in larval rearing of mud crab should be limited to the early zoeal stages. Experiments conducted in Australia (Rusoe et al 2004) indicate that although rotifers are essential for the acceptable larval survival, it can be removed from the feeding regime as early as third zoea. Artemia should be provided from second day of second zoeal stage.

Production of phytoplankton and rotifers should be synchronized with the hatching operation so that these are available as soon as the eggs hatched to zoea. Suggested feeding regime is given in the Table 5.

Water management: Water exchange is the mst economical method for keeping the good water quality. Water for rearing in treated with 10-20 ppm hypo chlorite and neutralized by strong aeration until chlorine residues have evaporated by addition of sodium thiosulfate. Water should be treated with 5-10 ppm EDTA to chelate heavy metals. For better results water is allowed to stand for three days after neutralization before this is used for culture. Rearing of water is replaced daily at 50-80% of the total volume starting day 2 or 3. Dead larvae and uneaten feeds are siphoned out prior to water exchange. Salinity of water may be reduced 32 ppt to 26 ppt starting zoea 4 until megalopa. In some cases rearing water is not changed but the volume is gradually increased as larvae grow. Once the megalopa is reached and water is changed almost daily from 30 to 50% of total volume. A few days prior to crab stage, net substrate and PVC cuttings are placed all over the tank bottom for attachment and refuge.

Nursery: Megalopa may be reared until the crab stage in the same larval rearing tanks or may be transferred to nursery tank and reared until 3-5 g prior to pond stocking. For the crab stage, 30-50% of water exchange is done daily.

Remarks: The larval rearing protocol for mud crab is still being refined by several organizations in India and elsewhere. It is hoped that further research will improve the technique so that cost of production will be reduced and commercial production of seed could be visible in near future.

Table 5 Suggested Larval-culture sequences and feeding and water management for mud carb hatchery operation

Day	Tank	Volume (L)	Stage	Chlorella Cell/ml	Rotifer Ind./ml	Artemia Ind. /ml	Water (%)
0	ST	300	Z1	-	-	-	-
1	LRT	500	Z1	50000	10-15	-	-
2	LRT	500	Z1	50000	10-15	-	-
3	LRT	500	Z1	50000	10-15	-	30
4	LRT	500	Z1	50000	10-15	-	30
5	LRT	500	Z2	50000	10-15	0.5-5	30
6	LRT	500	Z2	50000	10-15	0.5-5	30
7	LRT	500	Z2	50000	10-15	0.5-5	50-80
8	LRT	500	Z3	50000	-	0.5-5	50-80
9	LRT	500	Z3	50000	-	0.5-5	50-80
10	LRT	500	Z3	50000	-	0.5-5	50-80
11	LRT	500	Z3	50000	-	0.5-5	50-80
12	LRT	500	Z4	50000	-	0.5-5	50-80
13	LRT	500	Z4	50000	-	0.5-5	50-80

14	LRT	500	Z4	50000	-	0.5-5	50-80
15	LRT	500	Z4	50000	-	0.5-5	50-80
16	LRT	500	Z5	50000	-	0.5-5	50-80
17	LRT	500	Z5	50000	-	0.5-5	50-80
18	LRT	500	Z5	50000	-	0.5-5	50-80
19	LRT	500	M	50000	-	0.5-5	50-80
20	LRT	500	M	50000	-	0.5-5	50-80
21	LRT	500	M	50000	-	0.5-5	50-80

ST: Spawning tank

LRT: larval rearing tank

Z: Zoea

M: megalopa

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Bio-floc based intensive nursery and grow-out shrimp farming technology

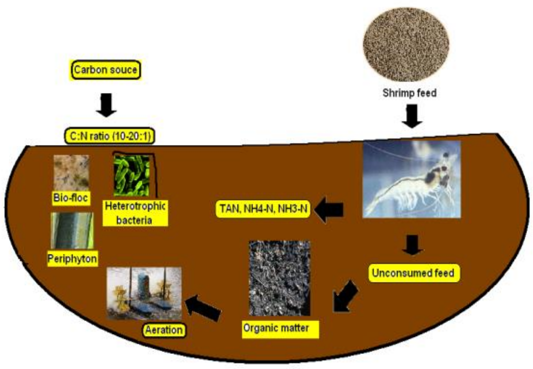
Panigrahi, A and Shyne Anand, P.S

Introduction

Improved and more cost effective aqua feeds and disease preventions are the major issues afflicting aquaculture industry for cultivation of shrimps/fishes causing loss of economic values in many ways. One potential approach for improving feed is the utilization of microbial communities as natural feed using nutrients available in culture ponds with sustainable mode. World aquaculture production continued to grow, reaching 82 million tonnes during 2019 of total fish production of 179 million tonnes (FAO 2020). In this connection, it is important to rectify, adapt and innovate new and sustainable aquaculture practices like Biofloc technology, which is changing the facet of intensive aquaculture with scope to attain high productivity with a sustainable approach. Bio-floc is the assemblage of beneficial microorganisms such as heterotrophic bacteria, algae (dinoflagellates & diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans & detritus. As it contains predominantly heterotrophic bacterial community over autotrophic and denitrifying bacteria, this can be controlled by maintaining high carbon to nitrogen (C:N) ratio. Biofloc in combination with periphyton (BPT) increases the natural production and in turn productivity. The concept of the retention of waste and its conversion of biofloc as natural food within the culture system is marked with lower/minimal water exchange, high density culture, reduction of feed and avoidance of disease outbreak (such as RMS, EMS, EHP) especially for the nursery phase of shrimp culture and so on.

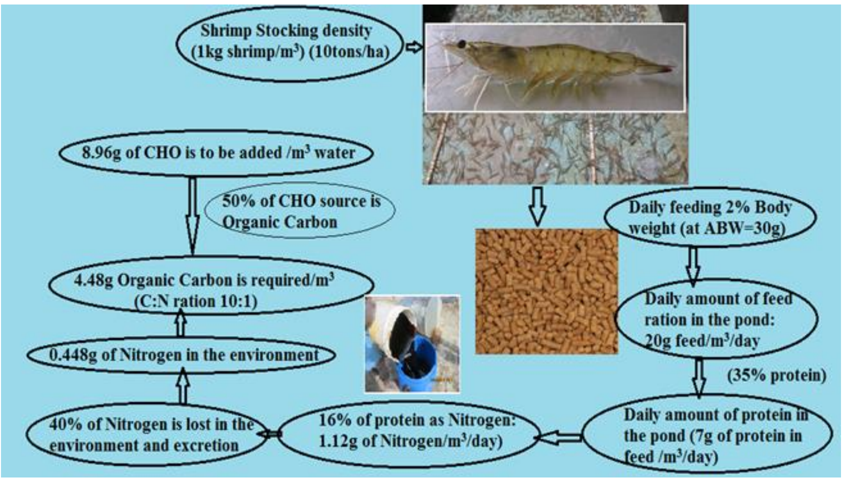
Principle

The biofloc principle combines the removal of nutrients from the water with the production of microbial biomass, which can be used by the cultured species, *in situ* as additional food source. Most importantly, microbial communities devour their augmentation from organic carbon (Schryver, 2008). Avnimelech (1999) calculated a carbohydrate need of 20g to immobilize 1 g of N, based on a microbial C/N ratio of 4 and a 50 % C in dry carbohydrate. The optimum C:N ratio in an aquaculture system can be maintained (C:N ratio 12-15:1) by adding different locally available cheap carbon sources and/or reducing protein percentage in feed. Under optimum C:N ratio, inorganic nitrogen is immobilized into bacterial cell while organic substrates are metabolized.



Evolution of Biofloc concept

As early as in the year 1976, Steve Surfling put forward the 'microbial soup' concept that eventually led to the development of "bio-floc" based aqua farming. After that BFT was developed at Ifremer-COP (French Research Institute for Exploitation of the Sea, Oceanic Center of Pacific) with different penaeid species including *Penaeus monodon, Penaeus vannamei*. Later, Prof. Yoram from Israel contributed immensely for the further modification and promotion of this encouraging technology. The technique developed in Israel subsequently spread to many other countries due to its several advantages.



Biofloc Constituents

In the floc 70-80% OM comprising heterotrophic bacteria, algae (dinoflagellates & diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans & detritus, though the composition changes rapidly and frequently through cycle, 40-50% of the pond bacteria is in flocs out of which >50% free living. Floc is 14-17 MJ/kg TE, 25-56% Protein, High levels of AAs esp. Lys, Arg, Leu, Thr, Val, Phe (but low Met), High (0.9) essential AA index, 25-29% organic C, 3-4% N, 0.5-12% high PUFA lipid, 22-38% ash, bioavailable organic minerals, vitamins, & enzymes and growth promoters (glucosamine)

Bio-floc based nursery rearing system

Nursery rearing of penaeid shrimps enhances the growth and survival of shrimps in grow out systems. BFT has been applied successfully in nursery phase in different shrimp species such as *P. vannamei*, *P. monodon*, and *F. setiferus*. Bio-floc and periphyton based nursery systems results in increases of 30 to 50% in weight and almost 60-80% in final biomass in shrimp at early post larval stage when compared to conventional clear-water system. Other advantages include better health and increased immunity through the continuous consumption of bio-floc which in turn positively influence grow-out performance. In a trial in India, the weight of the shrimp post larvae/ juveniles were enhanced from 15 to 250 mg with rearing densities above 10,000/ m^3 of water showing better performance in bio-floc system for *P. monodon* and *P. indicus* Nursery rearing under bio-floc system gives better result in terms of performance (growth and survival) and protective response.

Success of nursery depends on following criteria

- Properly designed nursery
- Healthy recruitment (Post larvae) - WSSV, EHP and IHHNV checking are mandatory
- Stocking density –optimum number in primary nursery phase depending on the species, infrastructure and requirements).
- In general, nursery systems may be one-phase, two-phase or multiphase operations.

One and two phase operations may combine outdoor and indoor systems. Primary nursery system (indoor) is referred as the extended larval rearing phase.

Advantages in nursery phase

- The primary advantage observed is related to a better nutrition by continuous consumption of biofloc, which might positively influence grow-out performance as compensatory growth phenomenon proved.
- Optimization of farm facilities provided by the high stocking densities in BFT nursery phase seems to be an important advantage to achieve profitability in small farms, mainly in cold regions or when farmers are operating indoor facilities.
- Study revealed that presence of bioflocs resulted in increase of 50% in weight and almost 80% in final biomass in pink shrimp, *F. paulensis* early post larval stage when compared to conventional clear-water system.
- Another study on *F. brasiliensis* post larvae reared with or without pelletized feed in biofloc conditions during 30-d of nursery phase, recorded 40% higher growth performance and survival compared to conventional clear-water continuous exchange system.
- For the biofolc generation in nursery system different inoculums like biofloc lyophilized powder or fermented biofloc product or recycled matured water can be used.
- Nursery rearing of penaeid shrimps at 5000-10000 nos/m2 could achieve 320-400 mg growth rate and 78-92% survival with 0.65-0.89 FCR in a month trial compared to 220-350 mg growth, 55-80% survival and 0.95-1.15 FCR recorded in biofloc based system.

Table 1. Growth performance of shrimps in Biofloc based nursery rearing system

Production performance	Control	Biofloc based nursery
Stocking (Nos/m2)	5000 to 10000	5000 to 10000
Floc volume	0-2 ml	3-12 ml
Nursery rearing days	3-4 weeks	3-4 weeks
Survival (%)	55-80 %	78-92 %
Average body weight at harvest	220-350 mg	320-400 mg
FCR	0.95--1.15	0.65—0.89

Source, GAA, 2009

Grow-out culture system with BFT/BPT

Similar advantage in bio-floc based grow-out systems has also been reported by many studies. As 20-30 % of the shrimp feeding is taken care by the floc particles, there is a potential gain in FCR. The selective breeding program for Pacific white shrimp, *P. vannamei*, requiring grow-out evaluation of selected families and involving the super-intensive shrimp culture with bio-floc has been conducted at Oceanic Institute in Waimanalo, Hawaii, USA, since 1997. These trials are conducted in a 75-m^3 super-intensive BFT raceway stocked at 300-400 shrimp/ m^3 in Oceanic Institute's Nucleus Breeding Center. In an experimental microbial floc culture system, shrimp given feed with less than25% crude protein performed similarly to shrimp raised under regular intensive culture with a37%-protein diet.

Water exchange

Zero water exchange makes the system more biosecured. In floc system, ammonia consumed by bacteria and nitrite increases, but tolerable at < 6ppm (15ppt), 15ppm (25ppt) & 25ppm (35ppt). May need to add hydrated lime/dolomite/bicarbonate due to declining alkalinity (120ppm declining to 20ppm at harvest). Alkalinity must be maintained >75-150ppm

Feed Protein level and Biofloc

The guiding principle is that around 95% of N added to pond is from feed/ferts and 50% of N in feed is released into environment. Must lower protein content to achieve good C:N ratio and reduce production of N-waste. At <25% feed protein, heterotrophic removal of TAN starts to dominate over autotrophic. This reduces feed cost (Ex. 30% Protien diet costs only 65% of 38% diet) = 35% reduction. *Vannamei* growth and survival equal or better at 20-

30% protein (7% lipid) than 40 % in commercial closed pond systems due to enhanced nutrition on flocs and reduced N-wastes.

Management

Floc management is basically done keeping in mind the needs of the bacteria and not the shrimp as total bacterial biomass is 2-5 times that of the shrimp. Floc volumes typically 2-4 ml/l first 2 months, then 6-20 ml/l later. Total soluble solids should be managed to be less than 300 ppm (3 mt/ha) to reduce aeration requirements. With C addition, TAN can be limited at 0.5-1 ppm. pH should be controlled by addition of lime /dolomite/bicarbonate (100-200 kg/ha/d until get alkalinity 75 ppm.

Carbohydrate addition

CHOs added to promote heterotrophic bacteria (HB) as these bacteria use organic C as energy source & uptake N to grow. Simple sugars like sucrose and molasses induce to grow the floc faster, however requiring frequent additions. In contrast, complex starches i.e. corn, cassava, tapioca, wheat & cellulose most stable but slow to react, can also act as bacterial substrates and contain suites of enzymes useful for digestion once ingested by shrimp. The lower the feed protein level, the less CHO required and requiring 20 ppm CHO (6g C) to remove 1 ppm TAN (100% removal) – produces 8g of microbial biomass & 10g CO_2 In another study using tilapia farm effluent, it was determined that 1 kg of microbial floc could be produced per 1.49 kg of sucrose. In minimal-exchange, intensive systems excess nutrients are assimilated and mineralized by a dense microbial community in the water column, thus alleviating potential toxicity.

Walking through Grow-out experiences

- It was estimated that more than 29% of the daily food intake of *P. vannamei* consisted of microbial flocs, decreasing FCR and reducing costs in feed.
- A 120 days trial of *L.vannamei* with different stocking densities, 150, 300 and 450 shrimp /m2 had a survival of 92, 81 and 75%, respectively. Moreover, study performed in a heterotrophic-based condition detected no significant difference in FCR when feeding *P. vannamei* with 30 and 45% CP diets and 39% and 43% CP diets, respectively in with and without floc system.
- It is noticed that glucose or a combination of glycerol plus *Bacillus* as a carbon source in bioreactors led to higher biofloc protein content, higher n- 6 fatty acids. It is also documented that no significant differences in growth performance of shrimps fed with 44% CPunder BFT and clear-water conditions.
- One of our studies conducted in ICAR-CIBA reveals the utility of reducing the protein in feed while customising feed for BFT.
- In one of the trial in Malaysia, where different stocking densities of 40,60,80,100 and 130 nos/m2 was tried in a biosecured biofloc module of HDPE lined ponds and yielded

a production level of 9.75 to 15.43 tonnes/ha/crop with FCR of 1.32 to 1.74 (Taw and Saleh, GAA 2013). Energy input was found to be 355kg/hp to 643kg/hp.

- Biofloc based recirculatory aquaculture systems conducted at Ocean Institute at Hawaii by Moss et al.(2006) recorded a productivity of 7.5 kg/m3 with an average body weight 24.7 g in a stocking density 300 nos/m3.
- When penaeid shrimps reared at 500 nos/m2 recorded a survival of 78-81% survival with a production performance at 8.86-9.2 kg/m3(Samocha e al., 2007).
- Recirculatory Aquaculture System conducted at Texas A & M University by Samocha (2009) recorded a final productivity 9.37 kg/ m3 with an average body weight 22.36 g from a stocking density at 450/m3.
- ICAR-CIBA static grow out system recorded a productivity of 5 kg/ m3 with an ABW 36-40g from a stocking density 200/m3.
- Growth performance of shrimps reared at biofloc based Hitide farms in TamilNadu recorded 39 g average body weight with 19.78 t/ha/crop compared to 15.7 t/ha/crop with 31.68 g ABW at 60 days of culture.

Research activities (ICAR-CIBA)

- At CIBA-ICAR achieved a number of milestones in biofloc and periphyton based nursery and grow-out farming technology. It includes characterisation of biofloc and periphyton, generation of biofloc, standardisation of various carbon sources, optimal Carbon Nitrogen ratio and optimal protein regime, and BFT based nursery and grow-out phases
- In the nursery phase, very high survival of 98-99% was achieved BFT system when compared to the conventional system (91-92%).In the grow-out phase, achieved a production level of 4 to 4.5 kg/cu. m (40 to 45 tonnes per ha) was achieved through this BPT system compared to 2.5 to 3 kg/ cu m in conventional autotrophic system (Panigrahi *et. al*, 2014). Effects of carbohydrate supplementation (Sujeet *et al*., 2014) and C:N ratio manipulation on water quality, microbial dynamics and growth performance in tiger shrimp was established.
- CIBA-ICAR is working on for the improvement of pond design and construction (central drainage system) and culture management practices, elucidate the composition of biofloc and their nutritive values, developing system to indoor and outdoor trial, reduce the cost of production, Scrutinizing aeration methods in congruence the oxygen requirements in the BFT system, customising the stocking density of nursery rearing and grow-out culture, assessing the disease resistance, immunity of biofloc reared animals and making guidelines of bio-security measures and pursue the BFT culture system

Experimental trial with different stocking densities of *L.vannamei* in partially fully lined ponds

Nature of the pond	HDPE lined + concrete bottom	HDPE lined + earthen bottom	Fully Lined pond
Stocking density/m²	160.8	160.8	111.1
Average size harvested (g)	25	20.3	33.3
DOC harvested	129	118	161
Survival (%)	88.1%	100%	85.4
FCR	1.56	1.4	1.9
Disease incidence	Vibrio loaded	WSV	--
Average daily growth(g)	0.193g	0.172g	0.20
Production/ha	34908kg	33,975kg	47,766kg
Production cost Rs	245/-	240/-	275/-

**Source, Anand et al, 2015*

Advantages of bio-floc based aquaculture technology

- Biosecurity of the system can be maintained with Zero/minimal water exchange system.
- Heterotrophic bacteria can reduce toxic metabolites (NH_3, NO_2)
- Easier management and environmental friendly approach (reduced protein requirement, fish meal usage and water/nutrient discharge), diurnal changes (pH, O_2, CO_2) in pond water is reduced
- Doubling the protein utilization as the shrimp use proteins twice - eat feed and then harvest flocs. Enhance digestion (with enzymes and growth promoters)
- Probiotic action - more diverse aerobic gut flora reducing pathogenic bacteria (*Vibrios*).
- Role in immune response by stimulating humoral and cellular immunity
- Reduced costs (15-20% lower cost of production) including 30-50% cost savings in feed
- Augmentation of natural food and improvement of FCR

- Reduced sensitivity to light fluctuation
- Major advantage growing shrimp in biofloc will not require of multiple external filtration. It reduces the start-up operational expenses
- The high protein-lipid rich nutrients in bio-floc, including fatty acids protects against oxidation, vitamins, phospholipids and highly diverse "native protein", could be utilized continuously and thereby help in building reserve energy, broodstocks gonads formation and superior reproductive performance.
- Bio-flocs can act as a natural probiotic which could act internally and/or externally against, *Vibrio* sp. and ectoparasites.

Limitations

- To sustain the bio-floc, high stocking density-biomass of shrimp is required.
- Since oxygen is very critical, aeration and energy cost increases
- Involved more technicality and understanding of the system
- Start-up time period required
- Limited progress due to lack of proper technical and infrastructural facilities and restrictions

Conclusion

Eco-based approach like biofloc technology will enable aquaculture practices towards an environmental friendly line bringing in an innovative strategy for disease prevention and discouraging antibiotic, antifungal chemicals and other medicines application. Bio-floc technology with biosecure modular systems may be an answer for more efficient, sustainable, profitable aquaculture production. This technology have the obvious advantage of minimizing water requirement, recycling *in situ* nutrients and organic matter and in turn improving farm biosecurity by exclusion of pathogens, augmentation of natural food and improvement of FCR, providing stress-free environment. Availability of natural food in the form of microbial bio-floc compensate for higher protein requirement of aquatic species. Though this technologies have potential to revolutionise the aquaculture sector. However, this is still in an initial stage and lots of research is necessary for its modification, standardization and implementation.

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Importance of live feeds for hatchery seed production

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Live feeds generally used for aquaculture purpose includes microalgae, copepods, artemia, rotifers, microworms, duckweeds etc. Among the live feeds used for larviculture purpose, the brine shrimp Artemia is the most widely used because of its practical convienience of hatching from commercially available dry cyst embryo (Artemia cyst). Microalgae are an essential food source in rearing larval stages of fin fish species (Tilapia, Milkfish, Cod, Halibut etc), shell fish species (Penaeus zoea stages), marine bivalve mollusk (clams, oysters, and scallops), marine gastropods (abalone, conch) and zooplankton species (rotifers, copepods, cladocerans and brine shrimp).

Microalgae

Microalgae are unicellular photosynthetic eukaryotes of major ecological and economic importance worldwide. Marine microalgae are the floating microscopic unicellular plant of the sea water which is generally free living, pelagic in the size range of 2 to 20µm. Many of the microalgae have immense potential in aquaculture as a means of feeding larvae or enriching zooplankton for feeding fish/shellfish larvae. In addition to protein and energy supply, they provide other key nutrients such as vitamins, essential polyunsaturated fatty acids (PUFA), pigments and sterols, which are transferred through the food chain. The most commonly used species in brackishwater aquaculture are diatoms (*Skeletonema* sp, *Chaetoceros* sp, *Thalassiosira* sp) flagellates (*Isochrysis* sp, *Tetraselmis* sp, *Chlorella* sp), *Nannochloropsis* sp, *Dunaliella* sp etc.

Microalgal culture Protocol

Various chemical media are available for indoor and outdoor cultivation. (Guillard's F/2 medium, Walne medium etc). For growth of microalgae in indoor laboratories certain factors are essential like, nutrients through specific media, light intensity (5000-6000 lux), aeration/agitation, temperature (24 ± 1^{0}C), CO_2 (for better growth) etc. The nutritional quality of microalgal biomass is directly related to the culture conditions. There are five different stages in the algal growth (Fig 1).

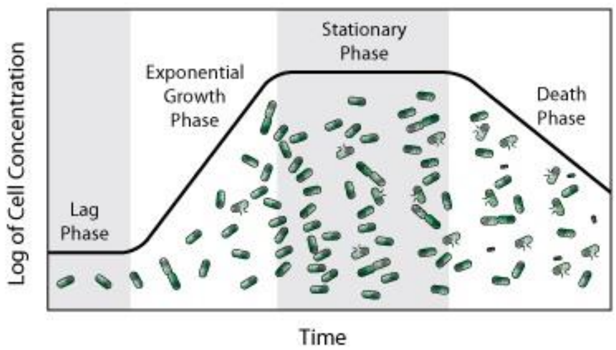


Fig1. Growth phases in the microalgal life cycle

Different types of cultures include:

Indoor/Outdoor

Indoor culture allows control over illumination, temperature, nutrient level, contamination with predators and competing algae, whereas outdoor algal systems make it very difficult to grow specific algal cultures for extended periods.

Open/Closed

Open cultures such as uncovered ponds and tanks (indoors or outdoors) are more readily contaminated than closed culture vessels such as tubes, flasks, carboys, bags, etc.

Fig 2: Indoor microalgal culture

Axenic/Xenic

Axenic cultures are free of any foreign organisms such as bacteria and require a strict sterilization of all glassware, culture media and vessels to avoid contamination. The latter makes it impractical for commercial operations.

Batch, Continuous, and Semi-Continuous

These are the three basic types of microalgal cultures.

Batch culture

The batch culture consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density. In practice, algae are transferred to larger culture volumes prior to reaching the stationary phase and the larger culture volumes are then brought to a maximum density and harvested. The following consecutive stages might be utilized: test tubes, 250 mlflasks, 3 and 20 l carboys, 500 l outdoor tanks, 5,000 l to 10,000 l outdoor tanks (Fig 3).

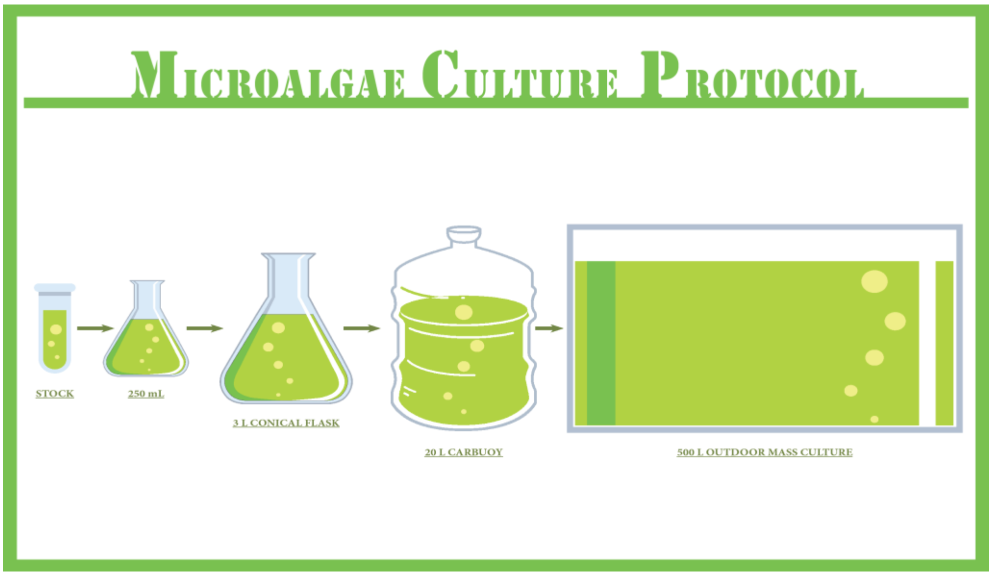


Fig3. Diagrammatic representation of batch culture of microalgae

Continuous culture

The continuous culture method, *i.e.* a culture in which a supply of fertilized seawater is continuously pumped into a growth chamber and the excess culture is simultaneously washed out, permits the maintenance of cultures very close to the maximum growth rate. Two categories of continuous cultures can be distinguished:

- *Turbidostat culture*, in which the algal concentration is kept at a preset level by diluting the culture with fresh medium by means of an automatic system.
- *Chemostat culture*, in which a flow of fresh medium is introduced into the culture at a steady, predetermined rate. The latter adds a limiting vital nutrient (*e.g.* nitrate) at a fixed rate and in this way the growth rate and not the cell density is kept constant.

Semi-continuous culture

The semi-continuous technique prolongs the use of large tank cultures by partial periodic harvesting followed immediately by topping up to the original volume and supplementing with nutrients to achieve the original level of enrichment. The culture is grown up again, partially harvested, etc. Semi-continuous cultures may be indoors or outdoors, but usually their duration is unpredictable. Competitors, predators and/or contaminants and metabolites eventually build up, rendering the culture unsuitable for further use. Since the culture is not harvested completely, the semi-continuous method yields more algae than the batch method for a given tank size.

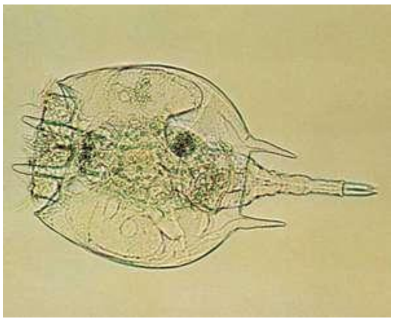
Algal production in outdoor tanks/ ponds

Large outdoor tanks/ponds either with a natural bottom or lined with cement, polyethylene or PVC sheets have been used successfully for algal production. The nutrient medium for outdoor cultures is based on that used indoors, but agricultural-grade fertilizers are used instead of laboratory-grade reagents

Nutritional properties of microalgae

The nutritional value of any algal species depends different factors, like culture conditions, phase of culture, contamination etc. Although there are marked differences in the compositions of the micro-algal classes and species, protein is always the major organic constituent, followed usually by lipid and then by carbohydrate. Expressed as percentage of dry weight, the range for the level of protein, lipid, and carbohydrate are 6.6-62%, 5.2-33%, and 4.6-23%, respectively. The content of highly unsaturated fatty acids (HUFA), in particular eicosapentaenoic acid (20:5n-3, EPA), arachidonic acid (20:4n-6, ARA), and docosahexaenoic acid (22:6n-3, DHA), is of major importance in the evaluation of the nutritional composition of an algal species to be used as food for brackishwater fish/shellfishes. Significant concentrations of EPA are present in *Nannochloropsis*sp, *Nitzschia*sp, *Chaetoceroscalcitrans, C. gracilis, S. costatum* and *Thalassiosirapseudonana* whereas high concentrations of DHA are found in the *Pavlova lutheri*,*Isochrysisgalbana* and *Chroomonassalina*. Micro-algae can also be considered as a rich source of ascorbic acid (0.11-1.62% of dry weight). Spirulina is a blue green alga with immense nutritional values. It has been declared a 'Superfood' for the 21st century by the World Health Organization.

Rotifer- as Live feed



Rotifers are important live food organisms (zooplankton) widely used in marine finfish and crab hatcheries throughout the world owing to their high nutritional value, small size and slow swimming behaviour which helps the early larval stages of fish and crab to prey on them. Most rotifer species are observed in freshwater environments, though some species are seen in saline water bodies. The phylum rotifera contains more than 2000 species of rotifers though the culture technology is available only for a few free swimming organisms. The most commonly cultured species of rotifers for which mass culture protocols are available fall in to the family, Brachionidae and genus Brachionus. These are euryhaline rotifer species with small size and a fast multiplication rate.

The most important rotifers species of commercial importance *is Brachionus plicatilis* (size: 150 -220 microns). Another important species of rotifers which is of great interest to aqua hatcheries is the smaller *B. rotundiformis* (size: 70-150 microns). The two species were earlier considered to be two strains of *B. plicatilis* and were called L strain and S strain. Modern taxonomy some time also classifies *B. plicatilis* as L type (large sized) and *B. rotundiformis* as SS type (small sized). The culture protocols for *B.plicatilis* or *B. rotundiformis* is one and the same.

Biology of *B. plicatilis*

Phylum: Rotifera
Class: Monogononta
Order: Ploimida
Family: Brachionidae
Genus: Brachionus
Species: plicatilis, (Muller, 1786)

Morphology, Feeding and Reproduction

Rotifers are also called *wheel animalcules*. *B. plicatilis* is morphologically divided in three parts namely head bearing wheel organ or corona, body forming lorica and a foot. *B. plicatilis* are filter feeders and feeds on particles less than 5 microns. They are non-specific filter feeders and some studies say that the particle sizes can be as high as 10 microns. Rotifers mostly feed on micro algae, though baker's yeast/marine yeast may also be provided. The most commonly used microalagal species for rotifer culture are *Chlorella*sp, *Tetraselmis*sp, *Isochrysis*sp and a few species of *Chaetoceros*. The species is dioecious i.e. sexes are separate. It resorts to both asexual reproduction (parthenogenesis) and sexual reproduction. The most common mode of reproduction under favourable conditions is parthenogenesis. The species has a life span of 5 to 7 days during which a female produces 18 to 20 eggs. Under favourable conditions of food availability the doubling time is 0.5 to 0.8 days.

Culture technique

Stock culture

Zooplankton sampling is carried out in salt water lakes or brackishwater bodies using plankton net of 50-100 micron capacity. The samples are observed under microscope and rotifers are picked up using fine dropper or micro pipettes and placed in to 10 ml test tubes containing algae @ 10 X 10^6 cells/ml. *Chlorella* or any mixed algae species of the stipulated size may be used as feed. Ensure that the salinity of the algal medium and that of the water samples from which rotifers where obtained are the same. Place around 20 to 30 rotifers in to the test tube. Scale up the culture through 50 ml, 100 ml flaks and then to 1 to 2 litre jars. Further scale up the culture to 20 litre buckets. The buckets may be used as inoculum for mass culture once the rotifer density exceeds 150nos/ml. The stock culture may be maintained in an enclosed room without any contamination. Air conditioning of the stock culture room may be avoided.

Mass culture

Batch culture

One tonne FRP tanks are used in this system of rotifer culture. The tanks are filled with filtered seawater to about 750 l. Inoculate the tank with two 20l cans of chlorella from indoor culture. The medium is then fertilised with the required salts. The chlorella bloom will be observed within 3 to 4 days. Rotifers is inoculated in to the tank when the algal cell density reaches 10 X 10^6 cells/ml. Rotifer inoculation is carried out using 20 l of rotifer stock culture with a rotifer density of not less than 150 nos/ml. The tank is fully harvested when the rotifer density in the whole tank reaches 100-150 nos/ml. The tank is then cleaned and the procedures are followed as mentioned earlier. The rotifer bloom is obtained normally by the 4^{th} day of inoculation.

Semi-continuous culture

The semi continuous rotifer culture is carried out in 5 tonne tanks. The tanks are filled with 2.5 tonnes of seawater and inoculated with chlorella. When chlorella bloom is observed 5, twenty litre rotifer stock cultures are inoculated in the tank. When rotifer density reaches 100-150 nos/ml by the fourth or fifth day of inoculation, 25 % of the culture is harvested on a daily basis. The reduced water volume is refilled with algal culture from algal culture tanks kept separately. The procedure is continued for 10 days after which the tank is emptied and a new batch is initiated. This is considered to a better model for a crab hatchery as crab larvae need to be fed with rotifers only for the first ten days of rearing.

Note

- Rotifer cultures often get contaminated with ciliates and in such cases the culture is discarded a new culture is initiated using a fresh and pure stock culture.
- In case of rotifer culture is contaminated with copepods, add chlorofos to the medium to kill the copepods.
- Dead algae in the tanks often flocculate and form suspended particulate matter which might seem to be rotifers. However, the best methods of ascertaining rotifer density in the tank is to take a 1 litre sample in a beaker and allow it to stand for 2 to 3 minutes, following which rotifer cultures will show a swarming movement and cultures with excess dead particulate algae will settle at the bottom or may appear the same without any swarming.

Artemia- as Live feed

Live food organism contain all essential nutrients includes protein, carbohydrate, lipid and amino acids commonly known as the "Living capsule of nutrition". A disease free healthy stock can be maintained by feeding with live food organism along with supplementary feed. Larval rearing phase is one of the most challenging phases in aquaculture. Artemia constitute one of the most extensively preferred live food item commonly used during the fin fish and shell fish larval rearing. It is commonly known as brine shrimp belonging to order Anostraca, class Crustacea and phylum Arthropoda normally found in natural salt lake or man-made saltern scattered throughout the tropical, sub-tropical and temperate climatic zones. The use of artemia in aquaculture includes it can be used for the larval and post larval stages of peneids, most successful diet throughout the larval rearing of fresh water prawn, used as the larval rearing of a number of marine fish species includes bream, bass, flat fish and also several species of fresh water and ornamental fish species. Artemia eggs contain 52% protein and 27% fat. Nauplii and Instar 1 contains 40% and adult contains 60% protein on dry weight basis, which makes them an excellent food for the larval stages. Due to its euryhaline nature, it can capable of withstand and reproducing in a salinity range of 5-200ppt. The main distribution of artemia in India reported from Tamil Nadu, Rajasthan, Maharashtra and Gujarat. The main advantage of using artemia is that it can produce live food on demand from dormant artemia cyst. Some of the strains are parthenogenetic (Only females) and most of them are zygogenetic (males and females). There are two modes of reproduction namely ovoviviparous in which free swimming nauplius get released by mother from the fertilized eggs under optimal condition and oviparous mode in which dormant cyst get produced under extreme or unfavourable condition.

Artemia cyst hatching procedure in indoor tanks

1. Place 5g of artemia cyst into 1litre of seawater in a hatching conical container along with continuous and vigorous aeration from the bottom.
2. Optimum temperature is 30^0C, pH of the water should be 8-9 and light intensity of 1000lux is required
3. After hatching of naupli within 24-48 hr, the air flow into the tank was turned off to let the tank settle for 10 minutes
4. The naupli attracted by light get concentrated at the bottom and harvest the nauplii by siphoning out and transferred to rearing tank enriched with organic matter, bacteria and algae.

Artemia cyst and biomass culture can be carried out in salt pans by fertilizing the ponds with both organic and inorganic fertilizer and inoculating the naupli which provide additional earnings to the salt pan owners.

Artemia production in salt pans (Outdoor culture)

1. Artemia production pond ($100m^2$) was dried, raked up properly to remove lab lab and other algal species
2. Sea water of 25ppt pumped into the pond using fine mesh screen. Water depth should be minimum 30cm and water temperature should not exceed 35^0C.
3. After one week of water intake, salinity was increased by gradual evaporation and fertilize the pond with inorganic (360g of Urea) and organic (7kg of dried chicken manure) fertilizer.

4. Weekly replenishment of fertilizer at the rate of 90g of urea and 1.8kg of dried chicken manure
5. Newly hatched nauplii get slowly released in to the pond and salinity gradually increased to 150%$_0$.
6. Cysts were collected regularly using fine meshed scoop net and washed with fresh water to remove soluble matters.
7. The cysts were transferred to a container filled with water of 250-300%$_0$ with continuous aeration. The cysts kept floating on surface and solid matter sink to the bottom.
8. Cysts were removed from the container and allowed to dry in air to about 10% moisture content level to avoid direct sunlight.
9. The dried cysts were transferred to air tight container and kept in a cool dry place.

Artemia biomass

Artemia is a cosmopolitan organisms distributed in tropical and sub-tropical zones. The brine shrimp Artemia (Crustacea: Branchiopoda) is a well-studied organism and it is a small shrimp like crustacean forms measuring 12mm (1.0cm) in length. It lives in hyper saline lakes, therefore, it is popularly known as brine shrimp. It takes only 15 days for the newborn larva to grow to adult size. The female adult Artemia releases 200-300 encyst embryos also called as Cysts (eggs). Artemia cysts have gained a unique position in aquaculture systems as they are highly nutritive, can be stored under ideal conditions for a prolonged period and hatched as and when required to get nauplii. Therefore, it is considered as an "Off and shell" on demand product for feeding early larval stages of cultivable crustaceans and fishes. *Artemia* is extremely important as standard live feed for over 85% of the marine aquaculture species (Kinne, 1977). *Artemia* is a biologically uncontaminated readily available and acceptable larval feed (Takami, 1993; Reddy and Thakur,1998) possessing several features, such as small size, easy ingestion (Leger *et al*., 1986), high nutritional value (Browne *et al*., 1991), unchanging food requirement from nauplii to adult (Helfrich, 1973) and high tolerance to various culture environments (Leger *et al*., 1987a).

Systematic Classification

Classification and taxonomy was first described by Scholsser in 1755 and renamed as Artemia (Leach, 1819)

Phylum	:	Arthropoda
Class	:	Crustacea
Sub-class	:	Branchiopoda
Order	:	Anostraca
Family	:	Artemiidae
Genus	:	*Artemia*

Subsequently, many population have been identified and currently the term Artemia is comprised of a complex of bisexual and parthenogenetic population by Browne and Bown and Sorgeloos, P 1993

Habit and Habitat

Artemia inhabits both inland and coastal saline environment, Manmade / managed solar salt work and Hypersaline lakes (100-350 ppt). In addition, it has high osmoregulating capacity -During low O2 level that prevails in high saline conditions. Non-selective filter feeder, tolerate wide range of salinity (5 ppt to 350 ppt) and temperature (6-35ºC) tolerance capacity. They are two types of population like Bisexual population and Parthenogenetic and also having two mode of reproduction such as Ovoviviparous (Nauplii production) and Oviparous – Cysts production)

Geographical Distribution

Distributed in widely discontinuous pattern and population are found in about 500 natural salt lakes of five continents. Among the bisexual strain, mainly six sibling species have been described so far Among the bisexual strain, mainly six sibling species have been described so far as Commercial species

1. Artemia tunisiana (Europe and North Africa)
2. *Artemia species* (America, part of Europe, Asia)
3. *Artemia franciscana* (America, part of Europe)
4. *Artemia parthenogenetica* (Europe, Africa, Asia, Australia)
5. *Artemia sinica* (Central Asia, China)
6. *Artemia persimilis* (Argentina)
7. *Artemia urmiana* (Iran)

Special characteristics of Artemia

- Wide range tolerance of physical parameters
- Non-selective filters
- Fast growth to reach adult
- Mode of population (strains)
- Mode of reproduction
- High range of fecundity
- Cysts (eggs) are viable for long periods
- Highly nutrients value (PUFA & HUPA)
- Enrich the nauplii (Bioencapsulation)
- Culture in traditional to super-intensive

Culture method

- ❖ Collection of *Artemia* Cysts and Biomass
- ❖ Cysts processing and storage
- ❖ Hatching techniques
- ❖ *Artemia* culture in laboratory condition, Algal culture maintenance

Artemia Cysts Hatching Techniques

Hydration

Take known quantity of Artemia dry cysts in a conical shape container containing Low saline water (15-25ppt) and provide vicarious aeration for 30 minutes, to Observe under microscope. Cysts should be in a spherical shape.

Decapsulation

Decapsultaion is essential process for disinfection for microbes, removal of external layer and improve the hatching efficiency. Take 5-10gms of hydrated cysts and transfer into a 500ml measuring cylinder, add 15ml of decapsulating solution with 85 ml of sea water. To provide vicarious aeration for 5-15minutes, when it will turn orange in colour, to be washed with running fresh water & incubate sea water for 24 hours.

Quality assessment of Artemia cysts

(i) Hatching percentage (HP)

250 mg of cysts (from each strain) were incubated in 80 ml of filtered sea water containing 30 ‰ salinity, pH 8.5 and temperature 29±1°C for 30 minutes and made up 100 ml in 200 ml measuring cylinder kept in standard hatching condition provided with mild aeration. After 24 hour of incubation, 5 sub samples of 0.25 ml were taken on to a petridish and the hatched nauplii along with unhatched cysts were fixed by adding two drops of Lugol's solution. The nauplii (n) and unhatched cysts (c) were counted under dissection microscope and the mean value was calculated by making use of the following formula.

$$\mathbf{HP\ \% = \frac{n}{n + c} \times 100}$$

Whereas	HP	=	Hatching percentage
	n	=	Number of nauplii hatched
	C	=	Number of unhatched cysts

(ii) Hatching efficiency (HE)

Hatching efficiency refers to the number of nauplii that can be produced out of 1-gram cysts under normal hatching conditions in 24 hours incubation period. 250 mg of cysts (from each strain) were incubated in 80 ml of filtered sea water containing 30 ‰ salinity, pH 8.5 and temperature 29±1°C for 30 minutes and made up 100 ml in 200 ml measuring cylinder kept in standard hatching condition provided mild aeration. After 24 hour of incubation, 5 sub samples of 0.25 ml were taken. From each, samples were pipetted out into a petridish and the hatched nauplii along with unhatched cysts were fixed by adding two drops of Lugol's solution. The nauplii (N) in each container were counted using a dissection microscope and the mean value was calculated as **Number of nauplii**

$$\textbf{Hatching efficiency} = \frac{\textbf{Number of nauplii}}{\textbf{One gram of cysts}} = \mathbf{N \times 4 \times 100 \times 4}$$

(iii) Hatching Rate (HR)

The hatching rate refers to the time period from start of incubation (hydration of the cysts) till the completion of the release of all nauplii. The following time interval can be worked out.

T_0 = incubation time till appearance of the first free swimming nauplii

T_{10} = incubation time till appearance of 10 % of total hatchablenauplii

T_{90} = incubation time all appearance of 90 % of total hatchableNauplii

T_s = T_{90} - $T_{10;}$ this value gives an indication of the hatching synchrony

250 mg of cysts were incubated in 80 ml of seawater in graduated glass cylinder with 5 replicates and made up to 100 ml. All containers were kept for incubation under normal hatching condition. After 9 hours incubation, 5 sub samples were taken from each sample at 3 hour intervals. Then the nauplii were counted by following the methods used for find the hatching percentage. The overall mean values were calculated for hatching rate.

Enrichment Methods

Step 1. Incubation of Cysts:

Step 2. After Artemia have hatched

Step 3. Collection the nauplii, wash in running sea water

Step 4. Heating of Menhaden Oil: Place 200 ml of menhaden oil into glass beaker and heat on hot plate to 50 C

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Step 5. Mixing of Ingredients: Pour heated menhaden oil into blender and add 200 ml of hot (40 to 50 C) tap water and mix.

Step 6. While mixing, add 10 ml of emulsifier and continue mixing until fluid turns into a creamy white solution.

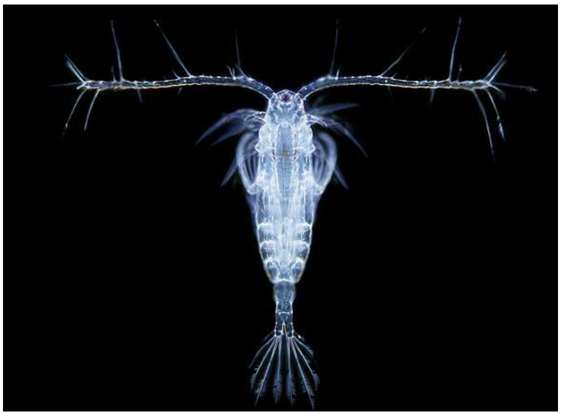
Step 7. Enriching Nauplii: The emulsion should be used at a concentration of 0.25 ml per liter of sea water

Step 8. Incubate the nauplii with the enrichment medium at a density of 300 - 400 nauplii/ml for a minimum of 6 h before feeding to fish larvae.

Step 9. Collect the enriched Nauplii and rinse thoroughly with sea water.

Step 10. Calculate the number of nauplii/ml as described previously and feed to fish larvae

Copepod-as Live feed



Copepods are small aquatic crustaceans inhabits a huge range of salinities from fresh water to hyper saline condition. It comprise of about 12000 described species comes under 10 order includes Platycopioida, Calanoida, Misophrioida, Canuelloida, Gelyelloida, Harpacticoida, Mormonilloida, Cyclopoida, Siphonostomatoida, Monstrilloida. Copepods are natural prey for finfish and shell fish larvae to meet larval nutritional requirements which improve growth, quality, increase survival rate and stress resistance. Copepods can be used as substitute of as supplement to artemia and rotifer diets serve as nutritional boost will have significant improvements on further growth pattern. Copepods eggs are readily be hatched into copepod nauplii (6 stages) size range from 50 to 700 micron with fed with micro algae, yeast, rice bran etc followed by sub adult and adult stages ranges from 700 to 1500 micron.

Mass culture of Copepod

1. Isolate one strain of copepod with egg from wild collected zooplankton

2. Put it in a test tube and fed with microalgae

3. Observe the test tube periodically and eggs hatches into Nauplius (1 to 6 stages). Fecundity may ranges from 12-45 nos

4. Pure culture can be transferred to conical flask and fed with microalgae, yeast, ricebranetc

5. Mass scale culture can be done in 500, 1000l and 5000l tanks with a density of 2000-4000 nos/l

6. Harvesting can be done periodically and feed to larval fin fish and shell fish species

Marine and estuarine polychaete worms as shrimp maturation feed

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Introduction

Aquaculture industry in the agriculture sector boosts the Indian economy. Disease-free healthy fish or shrimp are being produced by using commercial feeds. The enormous development of intensive shrimp culture in the recent years focused on the effects of waste water on the environment. Commercial feeds used in aquaculture will determine the production cost of fish, shrimp and also serve as fertilizers in grow-out ponds. These feeds can increase the waste load to aquaculture systems, if not managed appropriately which can impair water quality resulting in nutrient and organic pollution in water bodies (Tucker & Hargreaves, 2008). The commercial feeds are mostly applied in the system of mono species aquaculture systems, and their feed wastes are at times discharged without being used by other candidate fish species. They may either be assimilated by physical, chemical or biological processes within the grow-out ponds or discharged as effluents (Boyd & Tucker, 1995)

Aquaculture could provide food for the growing human population as well as reduce overfishing, nearly, one part of the global marine fish catch each year goes to feed other animals, because those wild fish can be acquired inexpensively from developing countries. Aquaculture must reduce its pressure on wild-caught fish for using as feed, as consumers concerned about health and the environment, we need diets that are more plant-based and fewer animals, but food production trends are not up to consumer demand alone. In the case of salmon fish, the consumers did not demand farmed salmon. Aquaculture could provide food for the growing human population as well as reduce overfishing, nearly, one part of the global marine fish catch each year goes to feed other animals, because those wild fish can be acquired inexpensively from developing countries. Aquaculture must reduce its pressure on wild-caught fish for using as feed, as consumers concerned about health and the environment, we need diets that are more plant-based and fewer animals, but food production trends are not up to consumer demand alone. In the case of salmon fish, the consumers did not demand farmed salmon. Of all the aquatic animal species groups that we farm for food, bivalves appear to be the most promising in terms of meeting these goals. Marine-based food such as squid meat, trash fish, clam meat, polychaete worms etc., are being continuously used as feed for aquaculture. Among the live fish food organisms, Polychaete worms may be a better option to feed brood shrimp or fish to facilitate their reproductive efficiency as they have more PUFA content. Among these, polychaete worms, *Nereids* in particular, are extensively used as live feed for shrimp and ornamental broods which enable successful breeding on a large scale (Olive, 1999)

Reproductive features of Polychaeta

Gambi & Cigliano (2006) studied the reproductive features of three species of Eunicidae (Polychaeta). The polychaete, *Perinereis* cf. *nuntia*, a tropical species endemic in Thailand, was cultured in captivity. The eggs and sperm from sexually mature (epitokous) *P*. cf. *nuntia*, were artificially fertilized, and settled into a sand bed about 30 cm deep at the nectochaete stage. The sand beds were supplied with seawater at 30 PSU and the nectochaetes reared for five months after which some adults were observed to become epitokes. The culture method yielded 3 to 4kg Polychaets at an atokous stage per m^2 of culture area (Poltana, 2007).

Polychaete worms as source of PUFA for shrimps

Lytle et al., (1990) reported that polyunsaturated fatty acids form the comparative tool in assessing maturation diets for *Penaeus vannamei*. Various commercial diets used for aquaculture have a high concentration of n-6 fatty acids because they utilize terrestrial plant resources to produce the diet. There have been limited reports of successful shrimp maturation using only commercial feedstock as maturation diet. In most cases, maturation occurs only after adding high concentrations of n-3 PUFA in the form of live marine organisms to the diet. PUFA as a comparative tool in assessing maturation diets of *Penaeus vannamei* was analyzed by Lytle et al., (1990). Giangrande & Petraroli (1991) reported the reproduction, larval development and post-larval growth of *Nainereis* laevigata (Polychaeta). Maturation diet based on fatty acid content for male *Penaeus monodon* broodstock reported by Meunpol et al., (2005). Prevedelli (2007) noticed the influence of temperature and diet on the larval development and growth of juveniles *M. sanguinea*. Rouse (1999) reported about concept of trochophore in ciliary bands and the evolution of larvae in spiraling metazoa.

Many reproductive biologists are fully depend upon the use of polychaete worms, squid and clam meat for the gonad development of shrimps in the commercial hatcheries of India. Polychaete worms used in hatcheries are mostly obtained from wild (Olive, 1993). The demand for polychaetes increased with the intensification of shrimp farming and as a result, the natural stocks are depleting gradually and thus, may no longer provide sustainable supply for shrimp hatcheries. In addition, the issue on biosecurity concerning wild polychaetes prompted the shrimp farmers to obtain polychaetes from reputable sources, thus the culture of Polychaete worm's gains importance. Collection of polychaete worm as bait is a disturbance for feeding shore-birds. The natural supply of polychaete worms has not been sufficient to meet the market demand and to a greater extent the collection of worms was perceived as a non-sustainable activity with potentially detrimental effects on the natural environment. Culturing of polychaete worms for commercial use is obviously a preferable alternative method instead of collecting from natural sources. Continuous collection of polychaete worms from the backwaters can result in a negative impact on tropical biota**.** Hence, grow-out practices of polychaete worms can provide an alternative way to improve mariculture practices and enhance livelihood of fishers**.** As polychaete worms are available, the use of squid and clam meat will be reduced in commercial shrimp hatcheries.

Polychaete worms as feed and for sea angling

Polychaete worms *could* become valuable resource in course of development of world aquaculture, since they can be a source of PUFA and other growth factors which are important for egg maturation in shrimps (Leelatanawit et al., 2014 & Olive, 1994). The stomach contents of benthic feeding fish species were examined and polychaetes were present to the extent of 38% to 88%. Polychaete worms contribute as feed for commercially important Crabs in South East Coast of India (Varadharajan & Soundrapandian 2013). The polychaete worms play an imperative role in the ecology of marine communities and few species are being used as live bait in the sea angling (Murugaesan et al., 2011).

Polychaetes as substitute of fish meal and fish oil

Polychaete worms are used extensively as maturation diet for shrimp broodstock due to their PUFA content that helps in enhancing reproductive performances particularly arachidonic acid (Meunpol et al., 2005) as well as reproductive hormones such as prostaglandin E2 and prostaglandin F2α. The potential use of polychaete worms as a dietary source of protein, lipid, amino acids and vitamins for current aquaculture species is huge, especially at a time when there is much interest in the uses of alternatives to fish meal and fish oils. The recognition of these values which may derive from the utilization of polychaeta biomass will inevitably lead to demands that could not possibly be met by collection of polychaetes from the natural environment. The culture of polychaete worms could also provide a means of supplying live feed to support other sectors of the aquaculture industry. This is especially true where polychaetes could be used for the improvement of diets in hatcheries for both finfish and crustacean. The successful development of a self-sufficient polychaete production industry will have other important effects. It will create an opportunity to supply materials for the expanding aquaculture industry and provide materials for use in many branches of fundamental polychaete research. In Western Europe, the natural supply of polychaete worms has not been found to be sufficient to meet the market demand and, to a greater extent, has been perceived as a non-sustainable activity with potentially detrimental effects on the natural environment (Olive,1993). The shortage of natural supply has also encouraged illegal or counter management bait digging activities

Polychaetes in the global market

In 2015, the five most expensive (retail price per kg) polychaete species sold in the global market were *Glycera dibranchiata* (US$ 237), *Diopatra aciculata* (US$ 150), *Nereis (Alitta) virens* (US$ 96), *Marphysa sanguinea* (US$ 85), and *Arenicola defodiens* (US$ 82) according to the three UK-based ragworm fisheries (Watson et al 2017).

Ecological importance of polychaetes

In the natural environment, they feed on detritus and smaller benthos and some species prey on other small animals using their retractable pharynx, and are in turn fed upon by higher order predators such as fish, crustaceans, larger invertebrates and even birds. The significant role played by polychaetes in nutrient cycling sustains the benthic environment. Head-down deep Polychaeta's are deposit feeders known for having strong effects on bioturbation and nutrient mineralization both by sediment ingestion, reworking and burrowing (Papaspyrou et al, 2007) and enhance organic matter mineralization and recycling.

Polychaete worms as carrier of viral pathogens

In recent years, however, the collection of Polychaeta's from the wild for aquaculture purposes declined due to biosecurity reasons as polychaete worms collected from the wild are possible carriers of pathogens as they accumulate the viral pathogen in their digestive tract by consuming virus particles in the sediment**.** When such worms from the wild are used as aquaculture feed, pathogenic diseases could be transferred to healthy broodstock shrimps leading to possible viral infection in the shrimps (Vijayan et al., 2005).

Import of polychaete worms

In many countries, including Asia, the main focus on polychaete worms is on the supply and export of *Nereids* worms. In Japan, the Nereids worms are imported from China. Polychaete fisheries is a thrust area in research and there is (1) a lack of knowledge of the biology and population dymanics (2) limited understanding of direct harvest as well as indirect impacts of harvesting (3) reliance on wild harvest with few cultured species (4) problem with bio-security associated with live exporters. Hence improved the understanding of the taxonomy, dynamics of polychaete population and their fisheries activities will support more effective and efficient management for Polychaete users (Cole et al 2018).

Diversity of polychaete worms

The diversity and spatial patterns of polychaete worms were studied in 28 stations by Sonia et al., (2009) covering subtidal and intertidal segments as well as builders and docks of the Mumbai Port. Ajmal Khan et al (2005) studied the diversity of estuarine polychaete worms from India. Thirty species of polychaete's belonging to eight families and 23 genera were recorded for the first time from the Andaman and Nicobar Islands, of which 15 species are new from Indian waters (Rajasekaran et al., 2012). A sudden and mass outburst of the epitoky polychaete worm *Nereis* (*Neanthes*) *virens* (Sars)/*Alitta virens* was observed of the surface waters of Middle Strait, Baratang, and South Andaman Island during July 2014. *A. virens* was studied for its morphology and structural characteristics and was the first recurrence in such a huge mass. These epitoky worms were observed in the month of July 2014 during monsoon season from the Andaman Nicobar Islands (Muruganantham et al., 2015).

Estuarine polychaetes in India

Information on polychaetes is available only from 8 estuaries out of 33 on the east coast and only from 4 out of 34 on the west coast and 153 species of polychaete's occurs in Indian estuaries, representing about 37.46% of the total polychaete's present in Indian seas. While 119 species were found to occur only in east coast estuaries, only 11 species were found in the west coast estuaries. Twenty-three species were found to occur in the estuaries of both the coasts. Besides increasing the extent of coverage on the distribution of polychaetes from other Indian estuaries, there is also a strong need to understand the variations in their diversity in relation with environmental changes, especially in terms of pollution (Ajmal Khan & Murugaesan 2005)

Swarming pattern of Polychaetes from Periyar river

The occurrence of red colored mass reproductive swarms of Polychaete worm, *Dendronereis aestuarina* from Periyar River of the south west coast of India has been reported by Jeyachandran et al (2015). Reproductive swarming behaviour of *D. aestuarina* has been first reported by Southern, 1921 from brackishwater environment in the Gangetic delta, however their report happens to be the first from freshwater environment. The density of swarmed polychaete was @14800 individuals/m^2 with the average length of 86 ± 16 mm. Male worms dominated in the 60 specimens analyzed and the sex ratio was 3:1 (M/F). The average diameter of eggs collected from polychaete body was 0.33 ± 0.08 mm. swarming was extended for two days; after successful mating and spawning they died.

Larval development of *Marphysa gravelyi*

The larval development of *M. gravelyi* (Polychaeta: Eunicidae) from Pulicat Lake, was studied by Malathi et al (2011). The adults of *M. gravelyi* have an elongated, reddish-brown body and live inside burrows in the muddy bottom of the lake. They produce benthic jelly masses in which the embryos occupy the core and are protected until the completion of larval development. Four different larval stages were observed: **proto-trochophore, early meta-trochophore, late meta-trochophore and nectochaeta.** These lecithotrophic larvae are found to be swimming inside the jelly mass. The larval development was completed in 8–10 days.

Development of commercial sand worm polychaete

The common polychaete worms used for shrimp hatcheries in Thailand are sandworms (*Perinereis* sp) and mud worms (*Marphysa* sp) (Meunpol et al., 2005). However, their available quantity and quality fluctuates with the season and the environment. In order to reduce the impact of over-harvesting, and to produce pathogen-free sandworms for shrimp hatcheries, Chunhabundit (1991) developed a semi-sterile technique for commercial sandworm farming.

Culture of *Marphysa* spp in indoor tanks

Marphysa sp. is commonly used as feed for shrimps and is abundant in mangrove wetlands and fish ponds in northern Iloilo, Philippines. This species belongs to family Eunicidae which is known to be gonochoric (with separate sexes), exhibit no sexual dimorphism (no difference in male and female physical attributes), and capable of multiple reproductive cycles throughout their lifetime (Gambi & Cigliano, 2006). The multiple reproductive strategies exhibited by *Marphysa* sp. is considered as a sustainable advantage over other polychaete species under the family Nereididae, in which death follows right after spawning (Fischer & Fischer, 1995).

Conclusion

Culturing polychaete worms and using as live shrimp feed will supplement suitable amino acid and fatty acids. Replacing of fish meal partially by polychaete meal and increasing the amino acid composition of new feed formulas by adding polychaete meals will be the new areas of interest. Modern aquaculture relies on pathogen free aquatic diet especially, live feeds, hence polychaete can be used to induce controlled spawning process in several fish and crustaceans species by supplying spawners with essential fatty acids and nutrients. Culturing of polychaete worms within land-based system without connection to the environment can provide pathogen-free (SPF) polychaete worms.

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Mitigation of microbial diseases in shrimp hatchery

Sudheer, N.S and Vinay, T.N

Introduction

Health management is the key to success and profitability in aquaculture. Adoption of proper health management strategy in shrimp hatchery could prevent the outbreak of microbial diseases. Major diseases that are important to the shrimp industry are of bacterial, viral, fungal, protozoan, and environmental etiology. Over the years, diseases are limiting factor to aquaculture productivity and profitability. Major important pathogens of shrimp include, Virus: White Spot Syndrome Virus (WSSV), Yellow Head Virus (YHV), Hepatopancreatic Parvo-like Virus (HPV), Monodon Baculo Virus (MBV), Taura Syndrome Virus (TSV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), Infectious Myonecrosis Virus (IMNV), Laem-Sing Virus (LSNV), Mourilyan Virus (MoV), and Shrimp Hemocyte Iridescent Virus (SHIV), Bacteria: *Vibrio parahaemolyticus, V. vulnificus, V. alginolyticus, V. campbellii* and *V. harveyi*, Protozoan: *Zoothamnium sp., Voritcella sp.,* and *Enterocytozoon hepatopenaei (EHP),* Fungus: *Lagenidium sp.* and *Sirolpidium sp.* The prevalence of the disease in shrimp culture systems is alarming such that there is a need for effective and sustainable control measure of disease outbreak. A better strategy to prevent the entry and growth of harmful organisms in aquatic systems is to control the environment through microbial manipulation.

Microbial management

Management of the culture environment is important for aquaculture production and profitability. Microbes present in the aquatic systems are either beneficial to the system or harmful. Microbial management includes preventing the entry of harmful pathogenic organisms into the culture facility and improving the growth and sustainability of beneficial microbes. The microbial growth and reproduction in the aquatic environment could be controlled through physical, chemical, and biological means.

Physical and chemical methods

Physical methods to control microbes in shrimp hatchery include Heating, filtering, Ultra vilot (UV) disinfection and Ozonization:.

Heating: Sun drying of equipments and other utensils is very common in hatcheries. Boiling or Autoclaving of water used for algal culture is effective. Small metallic equipments and glass wares could be disinfected in boiling water. The disadvantage of boiling is that it will not kill microbial spores.

Filtering: Filtering of inlet water through various types of filters is done in every hatchery. Filtering reduces the entry of vectors and microbs at certain extant. Most commonly used filters in the hatchery are filtering through sand filters (gravity or pressure) or mesh bag filters. Then pump to settling or into the reservoier tank. This is followed by primary disinfection and further filtered through a finer (1–5 µm cartridge) followed by UV. The use of activated carbon filter or biofilters will further enhance the water quality.

UV treatment: Ultraviolet radiation treatment is a widely used physical method in any water purification process. The UV radiation is effective against many bacterial species as it directly interacts with DNA replication in microbial cells via the creation of thymine dimers. For effective disinfection from viruses (including WSSV), bacteria, fungi, and protozoa UV irradiation must reach >30 000 mws/cm2 in the incoming water flow.

Ozonization: Ozone (O3) is a powerful oxidant, which is used along with the filtration system or RAS. It is highly effective in removing particulate and dissolved organic matter, disinfecting or reducing the microbial load in the culture water. For effective removal of viruses, bacteria, fungi from hatchery inlet water 0.5 µg/ml of ozone for 10 min exposure is required. The disadvantage of using ozone is its harmful effect and requires highly trained people to conduct ozonization. In seawater ozone results in the formation of hypobromite (OBr–) and bromate (BrO3–) which are toxic to aquatic organisms.

Commonly used chemicals used for microbial management in hatcheries can be broadly classified into disinfectants and therapeutics. Commonly used disinfectants are are sodium hypochlorite (Disinfection of water), benzalkonium chloride (BKC), calcium carbide, Potassium permanganate (Disinfecting broodstock) Na-EDTA, Hydrogen peroxide (Washing *Artemia* nauplii) Iodine compounds (Disinfection of egg) and zeolite (Water treatment), Hydrochloric acid (Disinfection of Equipments, filters, tanks etc) liquid povidone (PVP) iodine (Disinfecting broodstock,eggs, Nauplii). Theraputants: EDTA (Vibriosis) Copper sulphate (Filamentous bacterial disease), Treflan (Larval mycosis), Perfuran (Bacterial necrosis and *Vibrio* infection), formalin (egg washing) Malachite green for (Fungi and epibionts). Apart from these various antibiotics (Ciprofloxacin and Oxytetracycline) are also used to control microbial population. However there are strict regulatory measures implemented to restrict the use of antibiotics in many countries. Hatchery use of many of the antbiotics restricted or banned in India, hence antibiotic use in hatchery is not much discussed in this article.

Biological methods

Use of chemicals and other antibiotics is under restriction in many countries. Issues related to the deleterious effect of residual chemicals and antibiotics to the environment and concern over the generation of antibiotic resistance among bacterial pathogen is prompting to develop an environment-friendly microbial management strategy for aquaculture applications. Biological methods to control microbial growth in aquaculture systems include the use of probiotics, Bio-remediation, Bio-augmentation, Bio-stimulation, Recirculation Aquaculture System (RAS), and Phage therapy.

Probiotics

Role of microbes in controlling water quality in aquaculture is well documented. Probiotic organisms are microbes compete with bacterial pathogens for nutrients and inhibit the growth of pathogens. They are alternative for chemicals, antibiotics, and biocides used in the shrimp larval rearing facility. The application of antibiotics and other chemicals is restricted in most of the countries. The use of antibiotic for aquaculture is expensive, and excessive use of antibiotic will lead to the development of antibiotic resistance. The use of probiotic is much safer than antibiotics, and other chemical and prevalence of antibiotic residue in cultured shrimps could be reduced by using probiotic. Adition of probiotic bacteria in the culture system leads to stabilization of the exogenously added bacteria, and hence, it modifies the microbial community in the culture system. To achieve this, the probiotic should be applied at frequent intervals. Frequently used probiotic bacteria in aquaculture belong to the lactic acid bacteria (*Lactobacillus* and *Carnobacterium*), *Vibrio* (*Vibrio alginolyticus*), *Bacillus* and *Pseudomonas, Yeasts, Nitrosomonas, Nitrobacterium,* sulphide oxidizers. The probiotic could be used directly into the water or through feed /through live feed organisms such as *Artemia salina or Rotifer.* The mode of action of probiotics used in aquaculture are as follows: Production of inhibitory compounds (antibiotics, bacteriocins, siderophore compounds, lysozymes, proteases, hydrogen peroxide, and organic acids), competition for chemicals and growth factors such as iron, competition for adhesion site, enhanced immunity and improvement of water quality. Eventhough there were many commercial probiotic formulations available in the market, there are many concerns about the efficacy and safety of such products. This is because those products available in the market may contain ineffective bacterial species, due to unrealistic claims, lack of scientific evidence and poor quality control during production or inappropriate delivery methods leading to contamination or reduced performance.

Bio-remediation

The physiochemical and biological environment of the rearing system is important for the health status of the cultured organisms since aquaculture production results in the generation of waste materials. The wastes produced during larval rearing consist of metabolic by-products, residual food, fecal matter, and residues of prophylactic and therapeutic inputs. This condition may lead to the deterioration of water quality. Ultimately, it me end up with exposure of shrimps to toxins like hydrogen sulphide, ammonia, and carbon dioxide, which are stressfull to animals, immunity is weakened and result in disease outbreaks.

Bioremediation is an approach to improve water quality in the culture system through the application of microbes and their products such as enzymes that can degrade or utilize the toxic elements in the rearing water such as ammonia. Successful bioremediation leads to maintenance of low ammonia concentration, eliminate excess nitrogen as nitrogen gas, reduce the accumulation of hydrogen sulphide, minimize sludge accumulation, increase primary productivity. Bioremediation of organic detritus can be done through the use of genus *Bacillus* (*Bacillus subtilis, Bacillus licheniformis, Bacillus cereus, Bacillus coagulans),* and of the genus *Phenibacillus (Phenibacillus polymyxa).* These microbes are capable of producing a variety of enzymes that break down proteins and starch into small molecules, which are then taken up as energy sources by other organisms. The removal of organic compounds reduces water turbidity. The nitrogenous waste compounds such as ammonia, nitrate, and nitrite produced through fecal matter are bioremediated through ammonia oxidizers (*Nitrosomonas, Nitrosovibrio, Nitrosococcus, Nitrolobus,* and *Nitrospira*) and nitrite oxidizers (*Nitrobacter, Nitrococcus* and *Nitrospira*) and nitrifiers (*Pseudomonas, Bacillus,* and *Alkaligenes*). Bioremediation of Hydrogen sulphide produced in hatchery could be achieved by using photosynthetic sulphur bacteria that can break H_2S. Species such as *Rhodospirillum, Rhodopseudomonas, Rhodomicrobium, Chromatium, Thiocystis, Thiosarcina, Thiospirillum, Thiocapsa, Lamprocystis, Thiodictyon, Thiopedia, Amoebobacter, Chlorobiaceae, Chlorobium, Prosthecochloris, Chloropseudomonas, Pelodictyon,* and *Clathrochloris* are important photosynthetic sulphur degrading bacteria.

Bio-augmentation

Bio-augmentation is a kind of bioremediation process that involve the addition of microbes grown outside the system to enhance microbial populations at a site to improve clean up of toxic components. This process helps in reduce clean up time and cost. In this process, mostly, indigenous bacteria is preferred. Indigenous bacteria isolated through enrichment technique and grown in mas outside the site and applied in the site. *Bacillus sp.*is the most preferred microorganism in aquaculture use due to its ability to secrete multiple enzymes. However in recent past, *Paracoccus spp., Thiobacillus spp.* and *Aeromonas spp,. Acinetobacter spp,. Cellulomonas spp,. Nitrosomonas* and *Nitrobacter* and Bacteria strains belonging to the families *Chromatiaceae* and *Chlorobiaceae* are gaining importance.

Bio-stimulation

Improving the favorable environmental condition of naturally occurring bacteria to stimulate its activity growth is known as bio-stimulation. The process favor growth and reproduction of naturally occurring bacteria and hence its ability to degrade toxic waste. For example, mechanical aeration in larval rearing tanks not only provide aeration to cultured animals but also stimulate the growth of aerobic organisms that degrade organic matter and improve nitrification. Similarly, the addition of nutrients such as nitrogen and phosphorus to boost the capability of the microorganism of breaking down organic waste.

Recirculation Aquaculture System (RAS)

Adoption of RAS in shrimp hatchery not only reduce the use of water but also benefits by improving water quality that allows the growth of beneficial bacterial population. Adoption of RAS in shrimp hatchery provides bio-security and prevent the entry of pathogens. RAS is the best choice for broodstock management and maturation in hatcheries.

Phage therapy

Phages (bacteriophages) are viruses that invade bacterial cells, ubiquitous, obligate parasites, highly specific to their bacterial host. When a phage infects a bacteria, it undergoes either lytic infection cycle or Lysogenic pathway. During the lytic cycle, they multiply in the bacterial cell and lyse the bacterial cell at the end of the cycle to release newly formed phage particles. Lysogenic phages integrate its genome into the host as part of the host genome, replicate along with host genome and stay in a dormant state as a prophage for extended periods. If the host bacterium encounters adverse environmental conditions, the prophage may become activated and turn on the lytic cycle, at the end of which the newly formed phage particles will lyse the host cell. Phages are highly specific to their bacterial host. Nontoxic and nonpathogenic to higher animals and plants. Phages are highly specific and do not interfere with other beneficial flora in the culture system. Phages exist only when the host bacteria are present in the system. Different types of purified bacteriophage products as anti-infective agents include bacteriophage lysins and bacteriophage tail-like bacteriocins are used in phage therapy.

Conclusion

Controlling bacterial growth in hatcheries is an essential component in aquatic animal health management. Of the physical, chemical and biological methods are available for management of microbial growth in shrimp larval rearing system, biological means are highly valuable, since it is environment friendly and having various added advantages. Incorporating microbial management techniques along with strict biosecurity measures in hatchery operations results in sustainable environment friendly aquaculture production and profitability.

Concept of SPF, SPR and high health brood stock, in shrimp aquaculture

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Introduction

The global aquaculture production has increased significantly in last decade. Shrimp farming in brackishwater is one of the fastest growing aquaculture sectors in tropical countries including India. Brackishwater aquaculture in India is mainly dominated by the Pacific white leg (*Penaeus vannamei*) and in 2016, India became the largest exporter of shrimp and the sector is growing steadily. In order to meet the ever increasing demand for shrimp, the culture systems are getting highly intensified and to cope with the intensification, high quality seeds are required. This can be met by improving the growth and survival performance of the shrimp at adverse environmental conditions, by applying genetic selection.

Genetic selection can help in continuous improvement of plants/animals used in agriculture for better performance including growth, survival, disease resistance, color, feed conversion ratio etc. Over the last 40 years, the growth rate of broilers has increased over 400%, and studies have shown that 78% of this gain is due to genetic selection. Studies with shrimp have indicated potential gains of 5-15% per year. Although several shrimp breeding programs have been attempted, the most successful model is that of the Pacific white shrimp *(Penaeus vannamei*) which are subjected to long term family-level selection using specific pathogen free (SPF) base population. This approach has led to a healthy, fast growing population of Pacific white shrimp which have displaced much of the native shrimp farming in several countries including India.

Opportunity exists for further genetic improvement of Pacific white shrimp, specifically for selection of traits that perform well in specific local environments. In addition, similar breeding programs can be developed for other promising species such as black tiger shrimp (*Penaeus monodon*) and Indian white shrimp (*Penaeus indicus*) in India. Selective breeding requires that the lifecycle of target animals should be closed under captivity, i.e. animals should be able to live under human management from their birth/hatch onward and be able to breed and produce offspring that will survive to produce another generation of animals. Without this ability, broodstock needs to be taken from wild stocks every generation and selection of animals for improvement cannot occur. Hence, closing of the lifecycle under captivity is an essential part of selective breeding.

The breeding program should begin with SPF breeding populations housed within a Nucleus Breeding Center (NBC). The NBC is a biosecure facility isolated from shrimp farming activity. The facility is enclosed to avoid contamination and the buildings and water treatment systems are designed to facilitate proper sanitation and disinfection. Staffs are well trained in biosecurity techniques, and continual disease surveillance assures the SPF status of stocks. A typical breeding program starts with collection of wild broodstock and screening them for the presence/ absence of diseases and retaining only the disease free animals (Quarantine) to be the parent of next generation and get good quality SPF animals. These animals, after strict quarantine are moved into Nucleus Breeding Centre (NBC), where they are selected for desired traits, including growth, Specific Pathogen Resistant (SPR), uniform size, body color etc. The selected families are used for production of high quality broodstock at Broodstock Multiplication Centres (BMC) for propagation to different hatcheries, where there will be moderate biosecurity and once the animals are moved into commercial farms, the biosecurity level is low and the farming has to be done with all Best Management Practices to keep the shrimp healthy.

Brief description on the concept of SPF, SPR, NBC and BMC

What are SPF, SPR and SPT animals?

The concepts represented by the acronyms SPF, SPR and SPT have been used in a rather confusing manner over the last few years. SPF refers to the sanitary status of a stock, which means the stocks are free of certain pathogens, but not free from all pathogens, and not simply PCR negative. SPF stocks are free of certain pathogens regardless of its tolerance/resistance/susceptibility to any pathogen. SPR and Specific Pathogen Tolerant (SPT) refer to their genetic characteristics that allow them to be resistant to infection to a particular pathogen or tolerant to the development of the disease caused by a particular pathogen. These are genetic characteristics regardless of their sanitary status, whether the stocks are infected or not. In other words, stocks can be both SPF and SPR/SPT.

Nucleus Breeding Center (NBC)

Nucleus Breeding Centre is a facility where SPF shrimp broodstock are raised over a number of generations in highly bio-secured environment, excluding a number of pathogen of concern from the entire facility. A strict surveillance protocol is followed to ensure that the pathogens are excluded. The facility should strictly be situated where there is no shrimp culture activity in the vicinity.

Broodstock Multiplication Centre (BMC)

Broodstock Multiplication Centre is a facility, which receives SPF post larvae (PL) from the highly bio-secured NBC. The SPF post larvae are reared in BMC with a moderate bio-security, conducting surveillance to keep the pathogens away from the facility. The PL from NBC is reared to adult stage to produce high quality broodstock and is supplied to different hatcheries from here.

India being a leading shrimp producing country is yet to have a selective breeding program. However, the strong foundation has been laid to start a selective breeding program on Indian White Shrimp (*Penaeus indicus*) in coming days. At present, a BMC has been established by the Rajiv Gandhi Centre for Aquaculture (RGCA) at Mangamaripeta village near Bhimunipatnam of Visakhapatnam District of Andhra Pradesh. This is designed to provide consistent supply of High Quality SPF Pacific White Shrimp broodstock, that are selectively bred for good maturation performance, fast growth, resistance to diseases and high survival to the hatcheries in the country for production & supply of high quality seeds to farmers.

Import of *P. vannamei* broodstock and quarantine

Penaeus vannamei is an exotic shrimp species that has gained high culture momentum, since its introduction to India. Currently, the culture of the species in the Country is being done by the shrimp farmers by importation of Specific Pathogen Free (SPF) vannamei broodstock from approved suppliers, which are located overseas. The value of one brooder normally ranges from 50 to 61 US $, excluding the custom duty, processing fee and other charges for the transboundary shipment of the stock to India. The *P. vannamei* stock are permitted to be imported to the Country by the hatchery operators only through the single declared port of entry, i.e. Chennai in Tamil Nadu in the Country. The imported parent shrimps are then to be quarantined at the Aquatic Quarantine Facility before being transported to the vannamei hatcheries.

Suggested readings:

Hector et al., 2015. Genetic improvement of Pacific white shrimp [*Penaeus (Litopenaeus) vannamei*]: perspectives for genomic selection. *Frontiers in Genetics.* Doi: 10.3389/fgene.2015.00093

Gjedrem, T. and Robinson, N 2014. Advances by Selective Breeding for Aquatic Species: A Review. *Agricultural Sciences*, **5**, 1152-1158.

Gjedrem, T and Rye M, 2018. Selection response in fish and shellfish: a review. Reviews in Aquaculture (2018) 10, 168–179. Doi: 10.1111/raq.12154.

http://www.caa.gov.in/aquatic_quarantine.html

http://www.rgca.org.in/aqf.php

Overview of Eco-based production systems in India

Panigrahi, A., Biju, I.F and Arvind, R

Background: Low Input based Sustainable Aquaculture

The increase of productivity in aquaculture is accompanied by ecological impacts including emergence of a large variety of pathogens and bacterial resistance. These impacts are in part due to the indiscriminate use of chemotherapeutic agents as a result of very intensive management practices followed in production cycles. These impacts can be nullified through eco-based approach like organic aquaculture. Low Input Sustainable Aquaculture (LISA) 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 aquaculture production. It gives scope to the farmers to be innovative.

These organic production systems are based on specific standards precisely formulated for food production and aim at achieving agro ecosystems, which are socially and ecologically sustainable. The first “scientific” approach to organic farming can be quoted back to the “Later Vedic Period”, 1000 BC to 600 BC (Randhawa, 1986 and Pereira, 1993). The essence is to live in partnership with, rather than exploit, nature. This knowledge system is even today present with millions of Indian farmers as its practitioners and now with the increasing organic market, there is an organic revolution brewing throughout the country.

Main Features of organic farming

- Organic food production promotes biodiversity, biological cycles and biological activity.
- Organic farmers aim to manage food production as an integrated, whole system.
- Organic food production encourages the maintenance and sustainability of this system by restricting the introduction of harmful substances and practices that reduce, or alter the connectedness of the system’s components.
- Organic farming severely restricts the use of artificial chemical fertilisers and pesticides.
- Organic farmers rely on developing a healthy, fertile soil/water.
- Genetically modified organisms (GMOs) and products produced from or by GMOs are incompatible with the concept of organic production and consumers' perception of organic products.

Eco-based approach for brackishwater Aquaculture

Considering the full range of ecosystem functions and management the eco-based approach is traditionally practiced in brackishwater aquaculture in India. There has been research initiation to refine the technique and document the current practices. In this system effluents and residues from the farming system has been recycled and used as resources.

Traditional brackishwater aquaculture system in India

In the coastal states like Kerala, West Bengal, Karnataka and Goa, traditional brackishwater aquaculture prevails which are classical examples of integrated aquaculture, It is practiced in two forms 1) Temporal integration of rice with shrimp 2) Simultaneous integration of rice and fish culture. Chemical fertilizers or pesticides are not used. The economic return of rice-fish/shrimp integrated system indicates that rice and fish followed by shrimp provides significantly high economic returns. Presently, the traditional system is modified by stocking with hatchery reared seed and supplementary feeding. The recent research also attempts to use improved saline tolerant rice varieties to circumvent the low productivity of traditional rice varieties, to enable increased economic returns to the farmer (Sashidharam, *et al* 2012). The availability of hatchery produced seeds of penaeid shrimps and fin fishes such as sea bass, pearl spot and increasing knowledge about this ecosystem provides an opportunity to optimize the sustainability and economic viability of this type of farming practices. Traditional based aquaculture has evolved into an organised farm based aquaculture.

ICAR-CIBA Mission

Over the years ICAR-CIBA has generated significant information on hatchery and grow-out production, nutrition and feed-technology, disease diagnosis and management. These technologies have the ecosystem approach based footprints. CIBA have developed and demonstrated some of the eco- based system of farming based on LISA; like improved traditional system, Organic farming system; Substrate dependent (periphyton) and independent (Biofloc) based farming, brackishwater polyculture system, and integrated farming system involving rice, fish and horticulture.

Organic production system for brackishwater species

Organic aquaculture is a process of production of aquatic plants and animals with the use of only organic inputs in terms of seeds, supply of nutrients and management of diseases. Organic production system is an ecosystem based approach to aquaculture. Organic foods have a separate niche market and many farmers are attracted to these farming practices due to lower cost of production and better economic returns. Certified organic mussels, tiger shrimp, white shrimp, and tilapia also cultured in countries like Vietnam, Peru, Ecuador, Chile, New Zealand, and Israel.

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. It does not make any claims about the end product such as nutritional value or food safety; however organic producers have to follow the same strict guidelines at the local, state and federal level that all conventional food producers must follow.

International organic aquaculture standards

Other aquaculture standards have been developed, many still in draft form, throughout the world. These include Germany's Natural and, the UK's Soil Association, and Sweden's KRAV standards. The International Federation of Organic Agriculture Movements (IFOAM), a large umbrella organization, has also drafted organic aquaculture standards. The NATURLAND (Germany) and presently the EU regulations are more important for setting standards for organic shrimps production. The basics of organic shrimp production are: organic larvae, organic feed, stock density limit and certified packing plant in an eco-friendly operation. In India, INDOCERT provides certification for organic production systems.

National standards for organic production:

The standards and procedures have been formulated in harmony with the international standards such as those of Codex, IFOAM and keeping Indian requirements in mind. The guideline standard by NPOP under the apex body of APEDA, India is limited to the production, processing and certification of aquaculture. These standards shall apply to all aquatic organisms cultured in fresh and brackish water ponds and open water bodies in estuaries and sea. Organic producers must submit an organic plan which details all their management practices. They must maintain records to preserve the identity of the animals. They must document all of the feed sources and health care inputs and practices.

Although organic aquaculture is in a very nascent stage in India, its traditional system is close to the organic way of farming.

Organic shrimp farming Technology:

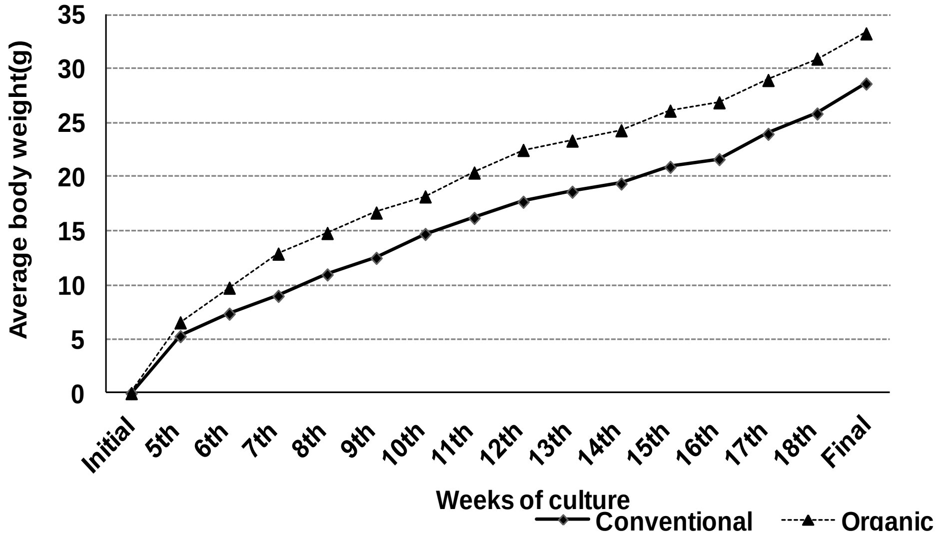
CIBA have developed and demonstrated a system of farming based on Low Input Sustainable Aquaculture (LISA). Like improved traditional system, Low input low cost system, Brackishwater polyculture system and integrated farming system involving rice fish horticulture. Besides these systems, the pond based organic farming of *Penaeus monodon* is developed with inputs like biocompost/vermicompost, yeast based organic preparations, and low fish meal feed. Effective utilization and exploration of natural productivity through organic manuring, zero tolerance to artificial fertilizer, pesticides, chemotherapeutants, medicines including antibiotics and integration of mangroves and other plants in the organic ponds were among some of the salient features of this farming practice. The following aspects are well-defined for initiating organic aquaculture.

- Conversion plan
- Organic inputs
- Sourcing of brood stock/ seed
- Organic feed development
- Farming procedures and processing
- Health management under organic banner

In our demonstration, the overall growth performance was better in organically managed ponds with a productivity in the range of 1200-1400 kg/ha. The success of this farming technology is marked with improvement in production level (14-21 %), size at harvest (10-19 %) with better FCR (lowered by 4-18 %) in the organic ponds. All these successful demonstration provide the baseline information to help formulate the guidelines for organic Aquaculture standard by APEDA, Govt. of India.

Feed developed with organic principle:

For the tiger shrimp and freshwater prawn, the fish meal content in the feed should not exceed 20 % and the total protein content of animal origin should not exceed 25%. The low fish meal based feed developed by CIBA avoiding incorporation of any antibiotics/ pesticides/ heavy metals/ antioxidants/ preservatives/growth hormones, adhere to the norms given by Nature land and other accreditation bodies with regard to the limit on the fish meal composition of the feed. The ratio of protein contributed from different sources (fish meal: other marine protein sources: plant protein sources) is 38: 35: 27 in control feed and 23: 24: 53 in low fish meal feed, thus keeping close to the guidelines.



Economics of organic farming:

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 indicated that there is a reduction in cost of production and increased gross and net returns in following organic shrimp farming compared to conventional shrimp farming in our farm. This is also true for other agricultural produce when it is done over the years. While standardising organic shrimp culture we had observed that major profitability comes from the reduced FCR compared to the conventional farms.

Challenges for organic aquaculture:

The challenges lies before in India are sourcing organically certified feeds or feed ingredients and producing organic brood stock and larvae. Further, closed reproduction of penaeid shrimps and antibiotic free hatchery and grow-out practices need to be achieved within a specified time as per certain standards. Marketing tie up with production which can help get the organic premium price needs to be channelized.

Aquamimicry based organic farming technology:

Aquamimicry technology is more recent phenomenon of organic farming following different strategy by supplementation of external carbon sources leading to the colonization of microbial/microalgal population which converts the harmful substances into consumable nutrient source which reduce the toxic deposition and also considerably reduced the need of water exchange. This technology is adopted to provide natural estuarine environment by promoting and balancing the natural planktonic community's growth. The culture environment preferably mimics natural estuarine environment with balanced water quality by acquired planktons. The use of feed and also water exchange can be reduced. The carbon sources serves as a feed for planktonic growth which served as feed for microbes and farmed shrimps. With supplementation of fermented rice bran at the rate of 500-1000 Kg / ha the pond the dominant growth of copepods within a week time is developed following which the post larvae are stocked (10-20 individuals/ m2). The fermented rice bran at the rate of 1 ppm will be added daily throughout the culture cycle for the purpose of maintaining the zooplankton as a feed. Later for feeding, fermented soy bean meal will be used which completely reduced the feed cost. Shrimp produced by this technology are red in color when cooked, likely from the consumption of natural foods that contain pigments (astaxanthin, amino acids and fatty acids, such as omega-3 fatty acids) which will add the marketing value as like "Organic shrimp" (Romano and Kumar, 2017).

Substrate independent (Biofloc) and dependent (Periphyton) based farming

Biofloc is the conglomeration of heterotrophic bacteria, algae (dinoflagellates & diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans & detritus. Biofloc technology (BFT) is based on the concept of the retention of waste and its conversion to biofloc as a natural food within the culture system. As the fish/shrimp ponds are rich in microbial community, the inorganic nitrogen added through the feed can be assimilated by these microorganism and converted in to microbial protein through an adjustment of C:N ratio. In a typical brackishwater pond, 20–25% of fed protein is retained in the fish/shrimp, rest is wasted as ammonia and other metabolites, Organic N in feces and feed residue. Increased C:N ratio through carbon addition enhances conversion of toxic inorganic nitrogen species to microbial biomass available as food for culture animals. Addition of carbon sources as organic matter substrate to allow aerobic decomposition and maintain high levels of microbial floc in suspension in feed and/or fertilized ponds. CIBA have successfully demonstrated the biofloc and periphyton based nursery technology (Panigrahi et al., 2018, 2019a,b) with very high survival of 98-99 % and grow-out farming in farmers pond.

Periphyton based farming is an attempt in this direction. Periphyton refers to the entire complex of attached aquatic biota on submerged substrates comprising phytoplankton, zooplankton, benthic organisms and detritus. The study conducted by CIBA (Shyne *et al* 2013) clearly indicates that periphyton has a beneficial effect on growth and production of shrimp. Better growth rate with a productivity of 1640 to 2796 kg/ha/crop at a stocking density 8-12 individuals/m^2 was observed. Further, the rate of return over operational cost was higher in periphyton-based system (92%) compared to the conventional farming (54%). This level of improvement of pond production with cheap on farm resources enhance the productivity of shrimp ponds without deteriorating ecosystem.

Probiotic based farming system

Antibiotic and other chemotherapeutics agents and also pesticides were commonly used in fish farms either as a feed additives or immersion baths to achieve either prophylaxis or therapy, Now a days, antimicrobial resistance is a growing public health threat and has been designated by the WHO as an emerging public health problem. Probiotic the natural beneficial bacteria are now well accepted and widely used in shrimp aquaculture. Antibiotics are used under the mistaken notion that they give better yield. Some hatcheries use banned antibiotics and other therapeutics causing environmental and health problems.

Polyculture based production system

ICAR-CIBA carried out several experiments to evaluate the production potential of poly culture of brackishwater fin fishes and shell fishes. In an experiment to evaluate the poly culture in an extensive system, farm level performance of two systems were evaluated: shrimp with mullets (*Mugil cephalus*, *Liza parsia* and *L. tade*), and shrimp milk fish (*Chanos chanos*). In the 180 day culture experiments, it was found that the production is similar in both systems. However, tiger shrimp out performed in mullet-shrimp system than the milk fish shrimp system (Biswas, 2012). It indicates that the mullet is more compatible with shrimp than milk fish. Further, this study also concludes that resource poor farmers can adopt this system as the input cost and expenditure is low.

Integrated multi-tropic Aquaculture (IMTA)

Integrated Multi-Tropic Aquaculture is the farming of different aquaculture species to gether in a way that allows one species' wastes to be utilized as feed for another. Farmers can combine fed aquaculture (e.g., fish, shrimp) with inorganic extractive (e.g., seaweed) and organic extractive (e.g., shellfish) aquaculture to create balanced systems for environment remediation (biomitigation), economic stability (improved output, lower cost, product diversification and risk reduction) and social acceptability (better management practices) (Barrington *et al*., 2009). This forming model can be developed for augmenting the average productivity of open water bodies.

Recirculatory Aquaculture System (RAS)

Recirculation aquaculture systems (RAS) developed as an environmental friendly novel indoor system for sustainable shrimp farming. RAS is an environment controlled system allows to rear shrimp at high densities, in indoor tanks. The considerable amount of water is re-used after mechanical and biological treatment and subsequently reduced the water and energy consumption. This will reduce the environmental discharge considerably and improved the access for waste management and nutrient recycling; moreover clear and sterile system avoids the pathogenic disease risks and thus allows to culture in high density rearing (Zhang et al., 2011)

For high density successful culture in RAS system needs continuous filteration and supply of clean water, maintenance of optimum temperature, dissolved oxygen is needed. It is not sure all the remaining orgnic matter could be removed by mechanical filter, the feed related waste outputs are discharged in the form of phosphates and nitrogen. Phosphates has no much toxic effect, but nitrogen in the form of ammonia giving adverse effect to the RAS reared animals. To avoid these circumstances, a biofilter system is necessary to purify the water and remove or detoxify harmful waste products and uneaten feed. Heterotrophic bacteria in biofilter oxidise the organic matter by consuming oxygen and producing carbon dioxide, ammonia and sludge. Nitrifying bacteria convert ammonia into nitrite and finally to nitrate.

Biofilters can be constructed using plastic materials with high surface area per m^3 of biofilter. This will allows the luxurious growth of bacteria as film so tight that it will get clogged with organic matter under operation. Also high level of free space for water flow with adequate back-wash give successful organic matter biodegradation. They can be fixed in submergible unit to improve the efficiency. Factors such as oxygenation with pure oxygen, ultraviolet light or ozone disinfection, automatic pH regulation, heat exchanging, denitrification, etc. depending on the exact requirements. Also nutritional fulfilment of candidate shrimp is taken into account for complete and success culture (Bregnballe 2010)

Aquaponics

Aquaponics is recent novel method combines aquaculture and agriculture. The nutrient rich discharges from regulatory system and other aquaculture system can be used to grow vegetable plants and other plants. The discharges from aquaculture units rich in nitrogen provides a natural fertilizer for the plants and the plants help to purify the water for the fish. This can be used to cultivate the vegetables in home level. The technology innovation will give the way to sustainable raise in commercial level. Aquaponics is easy to manage and providing fresh and safe vegetables free of pesticides, herbicides and chemical fertilizers. Successful aquaponics continuously recycles the water from aquaculture system and makes use as natural fertilizer for promoting the growth.

Conclusion

The ecosystem approach to aquaculture is mainly focused on low input based simple technology suited to the local conditions, providing sustainable, economically viable and socially acceptable models. India has vast resources of traditional farms which are close to nature which can be easily modified to suite these technologies. Most innovations and development of brackishwater aquaculture showcasing the economic earnings is mainly due to the “industrial” aquaculture, using SPF seeds, formulated extruded feeds, aeration and with the use of various pond management inputs. Balanced growth of these different trajectories on complementary and integrated mode is the need of the hour. Eco based innovative technologies like Biofloc or periphyton will certainly ensure to develop ecologically integrated aqua farming systems that are community-based, sustainable, and economically viable, along the side of industrial farming sector. The greatest constraints faced by transitioning farmers are the lack of knowledge, information sources, and technical support. Greater government investment in appropriate research and extension services can help overcome these constraints.

Probiotics based shrimp production system for shrimp health improvement and profitability

Panigrahi, A and Sudheer, N.S

Introduction

Based on the heightened expectations and enthusiasm of the shrimp farming sector in our country especially after the introduction of *P. vannamei* and revival of shrimp farming sector , hatchery operators have a pivotal role in providing adequate supply of healthy & disease free post larvae to the farmers. At present, there are about more than 250 numbers of shrimp hatcheries in our country with a production capacity of 11 billion post larvae per annum. Again for eco hatcheries aiming at organic and/or certified best quality seeds, zero tolerance for antibiotics and other chemotherapeutics are highly suggested.

Hatchery systems are often encountered with several disease conditions and are often enduring significant economic losses over the last decade, largely from viral and bacterial diseases, with a production loss of up to 100%. Rapid and sensitive disease diagnosis, prevention and control measures lag significantly behind even though shrimp aquaculture has made rapid advances Antibiotics are generally used to prevent the bacterial infection. However, antibiotics not only develop resistant strain creating health hazards but also are proven to induce immune-suppression in shrimps. Even though large quantities of antibiotics are being used in hatchery systems, in many cases, they become ineffective either because of the development of resistant bacteria or by the entry of pathogenic bacteria from other sources into a particular larval phase where they become insensitive to antibiotics. For example, resistance against Oxytetracycline, the most commonly used broad-spectrum antibiotics in hatcheries is increasing, and therefore treatment seems to be more often inefficient (GESAMP, 1997). Moreover, there is an increasing concern over the indiscriminate use of antibiotics, which can lead to alterations in the existing genome of bacteria and can even adversely affect microbial biodiversity. Hence, hatchery operators and farmers are still in the light of limitation to find a solution for the health problems which is currently plaguing the shrimp farming industry. This situation demands cost-effective and environmentally safe approaches, such as use of immunostimulants and probiotics (Pro: for & biotics: life), which is increasingly important to maintain a healthy microbial environment in the larval rearing systems. Hence the probiotics are promoted as an alternative health management tools in many fields including shrimp hatcheries.

Probiotic bacteria in disease control

Elie Metchnikoff described probiotics as the microbes ingested with the aim of promoting good health. This same definition was modified to as organisms and substances which contribute to intestinal microbial balance by Parker, 1974, and later by Fuller 1989 as live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. Taking into account the probiotic use in aquaculture, Verschuere et al. (2000) defined probiotics as “a live microbial adjunct which has a beneficial effect on the host by (i) modifying the host-associated or ambient microbial community, (ii) ensuring improved use of the feed or enhancing its nutritional value, (iii) enhancing the host's response towards disease, or (iv) improving the quality of its ambient environment”.

Thus the use of probiotics or beneficial bacteria to displace pathogens either by competitive process or by the release of growth inhibitors is now gaining acceptance in hatcheries as a more effective alternative to antibiotic administration to boost the health status of the larvae. Various studies clearly demonstrated the enhanced survival and growth rate of shrimp through the probiotic application (Maeda, 1994; Austin et al., 1995, Azad et al. 2005). Most widely used probiotic bacteria in shrimp larviculture and fish hatchery are

Lactobacillus spp. (Gatesoupe, 1991; Garcia-de-la-Banda et al., 1992)

Bacillus spp. (Rengpipat et al., 1998, 2000)

Vibrio spp. (Riquelme et al., 1997; Ringø and Vadstein, 1998)

Many investigations (Moriarty 1998 and Rengpipat et al. 1998) demonstrated that probiotics can be effectively used against luminous Vibriosis which, is a major problem encountered in hatcheries and can cause significant mortalities within 48 h when the bacterial count in larval rearing water increases in the range of 10^2–10^4cells/ml. There are various studies those shows the effective role of probiotics in health management of fish and shell fishes (Karunasagar, 2001, and Panigrahi, et al. 2005, 2007).

Probiotic component as immunostimulants

The Defense mechanism of crustaceans depends on the nonspecific innate immunity and in general, they lack specific immunity. Various findings indicated that it is possible to stimulate the natural immune system of crustaceans. Various microbial cell wall components such as β-1, 3/1, 6-glucans, peptidoglycan, Lipopolysaccharides etc. could be used as immunostimulants. Immunostimulants are described as substances that can boost natural defence system of the animal. Many of the immunostimulant molecules derived from bacteria, fungi or algae are now globally used to stimulate the non-specific immune system in shrimps. Even though, immunostimulants are used as prophylactic agents via formulated diet in grow out, its efficacy in the larval culture system is likely to be less as larval cycle of shrimp exclusively depends on live food organisms. Hence, it is necessary that the application of bio-encapsulated probiotic formulations instead of direct delivery. It is possible to bio-encapsulate probiotic organisms such as yeast (*Saccharomyces cerevisae)* in the live food organisms like rotifer *Brachionus plicatilis or artemia* (which are able to brake the yeast cell wall, allowing exposure of the potent immunostimulants β-1,3/1,6-glucans).Studies show that application of β-1,3/1,6-glucans to larvae in the early stage act as an alternative immune enhancer by improving the survival of post-larvae and the subsequent immune responses via activation of granular haemocytes of juvenile shrimp when challenged with WSSV (Chang et al., 1999, 2003, Rodriguez et al., 2007).

Probiotic as nutritional immunomodulator

Probiotic organisms are found to be the richest sources of many beneficial products, such as vitamin, minerals trace elements, and digestive enzymes that provide additional nutritional benefits. Feeding shrimp with probiotic enriched (*Bacillus spp*) artemia showed increase in nutritional efficacy. Other commonly used feed probiotics contain *Lactobacillus* sp., or *Saccharomyces cerevisiae*. These are reported to give better survival and growth and improve the protective response, especially during the larval stages. Cultures of live beneficial bacteria, when added to the larval diet, is found to discourage colonization of pathogens in the gut and there by overall improvement of nutritional status of the larvae.

Probiotic community for water remediator

The success of any hatchery system demands a continuous supply of pathogen-free good quality water with optimum water quality parameters. In spite of the elaborate water treatment system consisting of sand filters, cartridge filters and ultraviolet treatment, often the hatchery suffers from disease outbreaks. Probiotics can be applied in hatchery after proper water treatment which would facilitate the establishment of the beneficial bacterial population prior to the establishment of any pathogenic bacteria .As we know, water used in all the stage of hatchery operation is high in sediments due to suspended un eaten feed and faecal matters which can lead to the proliferation of pathogenic bacteria such as *vibrio* species. Hence periodical application of probiotics can prevent the pathogenic bacteria establishment and also improve the water quality by consuming decayed and suspended organic matters. The water probiotics contain multiple strains of bacteria like *Bacillus acidophilus*, *B. subtilis*, *B. licheniformis*, *Nitrobacter* sp., *Aero- bacter* sp., and *Saccharomyces cerevisiae*. However probiotic treated conditioned water must be used within two to three days of application. Periodic monitoring of water quality parameters and the microbial load is necessary to maintain healthy hatchery system.

Since, continuous water exchange can always increase risk of disease occurrence, presently, hatchery operators prefer minimum water exchange or recirculatory system supported by Bio augmentation and bioremediation technologies. Use of biofilms and periphyton-based complex microbial systems in shrimp hatchery system without water exchange are found to be effective to maintain low levels of ammonia and nitrite as these systems harbor a variety of beneficial probiotic and nitrifying microbial communities. These microbial communities play a major role in maintaining stability in water quality parameters and control of microbial populations. Not only these biofilms provide a suitable environment resulting in high health, growth and survival of larvae, but also minimize water exchange and decrease the risk of disease occurrence. There are various studies elucidating the beneficial use of bacterial preparations (bacterins) in crustaceans with highly encouraging results (Itami et al. 1989, 1991; Karunasagar et al. 1996; Devaraj et al. 1998; Azad et al. 2005).

Mechanism of action of probiotics

Acceptance of microbial intervention concept by regulatory bodies will be possible only if the mechanisms of their action are well explained. Probiotics acts in different ways and various proposed mechanisms for the mode of action of probiotics are given below.

- Antagonism (By affecting the balance between pathogenic and harmful microbes and beneficial microbes).
- Competition for adhesion sites and nutrients with other microbes in the niches, for chemicals and available energy
- Production of inhibitory compounds in host or in culture medium
- Improvement of water quality - probiotic bacteria can directly take up or decompose the organic matter or toxic material and improve the quality of water by Increasing DO, preventing off flavour, reducing N & P level, reducing BGA etc.
- Interaction with phytoplankton and improving natural productivity
- As a source of macro and micro nutrients
- Enzymatic contribution to digestion - The microbial cultures produce a variety of enzymes like amylase, protease, lipase, xylanase, and cellulase in higher concentrations compared to the native bacteria.
- Immunomodulation (Microbial products eliciting immune response at humoral, cellular and molecular level) results in Improving the animal's overall health by the possible exclusion of pathogens from the production system, Enhancement of animal's physical condition, appearance of average size and weight of the animal and restoration of reduced appetite and feed consumption.

However, modes of action are sometimes circumstantial and based on empirical arguments. The paradox concerning whether or not inactivated cells should be regarded as probiotics or even as oral vaccines exist in spite of a clear benefit using even dead cells is observed.

Probiotics and immune mechanisms

It is imperative to know the molecular aspects of host–probiont interaction in shrimp to engineer probionts for harvesting effective immunomodulation in the hatchery system. Non-specific defense mechanism of shrimps response to pathogen intrusion via pattern recognition proteins (PRP) (Soderhall et al., 1999).These proteins recognizes the conserved polysaccharides present in different probionts namely, β-1,3-glucan (βG) from yeast, lipopolysaccharide (LPS) from Gram-negative bacteria, and peptidoglycan (PG) from Gram-positive bacteria, and Gram-negative bacteria and in turn trigger cellular and humoral responses. The major proteins that function as PRPs in crustaceans are

(1) β-glucan binding or recognition protein (βGBP, βGRP)

(2) LPS and glucan binding protein (LGBP)

(3) Gram-negative binding or recognition proteins (GNBPs or GNRPs)

(4) Peptidoglycan recognition protein or peptidoglycan-binding protein (PGRP or PGBP)

It is known that the PRPs, like β GBP, LGBP, and PGRP which recognize and respond to microbial intruders, are involved in activating the innate defense mechanism especially the humoral defense cascades in shrimp. These responses are nonspecific and target a broad range of bacterial and fungal invaders**.**

Mode of application

There are three widely used routes of drug delivery in fish viz., injection, immersion and oral. In aquaculture, probiotics can be administered either as a food supplement or as an additive to the water. But in hatcheries preferred mode of application is immersion method or bioencapsulation of probiotic components through live food organisms. In hatchery systems, its application can start in the early stage of water treatment to prevent the proliferation of pathogenic bacteria and as water conditioner agent via immersion method.

Probiotics as a feed can be supplied from protozoea stage onwards along with the microalgal feed. Protozoea being filter feeding organisms can ingest beneficial bacteria in the rearing systems. In mysis stage either bioencapsulation of probiotic bacteria in live food organisms like artemia and rotifer can be done with or can be directly incorporated into microencapsulated diets. In the later stage of larval life cycle mainly post larvae cell wall component of probiotic bacteria like peptidoglycan, LPS and yeast beta glucan can be incorporated directly into formulated diets.

Factors such as growth and multiplication rate, revival percentage after storage, the effect on the host immune response under both stress and stress-free condition, efficiency in improving the water quality are to be studied before applying into hatchery systems.

Factors affecting the performance of probiotics *in situ*

The success of *in situ* performance of probiotic in hatchery system depends on many extrinsic and intrinsic factors.

The abiotic variables such as

Water temperature: (one of the important factors for selection of probiotic bacteria for aquatic animals which are essentially poikilothermic)

Dissolved oxygen: (Faster and more aggressive bioremediation happen in presence of optimum oxygen),

Salinity: (As the microbes differ in their affinity towards salinity and ion concentration) **Nutrients:** (organic substances as both carbon and energy sources),

Oxido-redox potential: (determines the nature of the microflora it can support and the metabolic product thus generated}, and

Biotic variables: (chlorophyl a, phytoplankton populations) are the extrinsic factors which can directly determine the growth and multiplication of the probiotic bacterial community. The food web interactions and stochastic population dynamics are important intrinsic factors affecting the affect probiotic performance. Thus appropriate biotherapeutic agents should be used depending on the abiotic and biotic factors of the larval rearing system.

Other Microbial products available in the market

Many of the microbial products available in the market are derivatives or cellular components of bacterial, fungal, or animal origin. Some of these products are listed below (Newman and Deup-ree, 1994).

Laminarin,	zymosan,
barley glucan,	inulin,
lactoferrin,	chitosan,
levmisole,	beta glucans,
lipopolysaccha- rides,	dextran,
curdlan,	lentinan,
scleroglucan,	peptidoglycons,

These products available in the market are under the brand name as Probact, Probacillus, Superbiotic, Super P S, Zymeline, Probioenzyme, Eudulact etc and the classes of bacteria found in these products are as follows; Gram-positive like *Arthrobacter*, *Bacillus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Micrococcus, Streptococcus and Weissella*, Gram-negative bacteria like *Aeromonas, Alteromonas, Photorhodobacterium, Pseudomonas* and *Vibrio* has been evaluated for probiotic applications.

- Rational selection and validation of promising microbial strains/products should be based on evidence obtained from experiments with *in vivo* and *in vitro* set up
- Strict regulation against the use of antibiotics and encouraging the environmental friendly microbial interventions
- Microbial management should be an integral part of all aquaculture practices
- Microbial product should be screened and evaluated for their efficacy in the field condition before marketing and also in process quality assessment should be done

- Awareness campaign to be organized to get rid of all antibiotics and tips to use microbial product for better productivity

- More information on efficacy and economics is required to be looked in to.

Parameters / Indicators to evaluate a product's efficiency

It has been for quite some time that probiotics play important roles in overall shrimp culture practices. Even though the probiotic application is one of the eco-options, when to and how to apply is the pertinent question. Hatchery operators often go for various probiotics and immunostimulant products from the market out of desperation than by established or researched technologies. Most of these so-called 'products' lack scientific evaluation. Inconsistent results, high claims, uncertain screening and regulation by authority sometimes put a question mark over its efficacy.

As we know, positive aspects of microbes in hatchery system are larger; microbes may cause significant losses to operations if they are pathogenic. For example, the use of vibrio species as probiotics in aquaculture remains controversial, because certain genus used has been found to be pathogens. In Ecuadorian shrimp hatcheries *Vibrio alginolyticus* is observed in both, healthy and non-healthy larvae cultures (Vandenberghe et al., 1999). Hence, an effort to compile the disparate but relevant facts about the use of microbes in hatchery systems is essential.

Hence efficiency of bacterial amendments can be questioned if they are not using it judiciously. Rather, the hatchery operators need to adopt strict biosecurity protocols along with prophylactic agents like probiotics to ensure its future. Thus sustainability and profitability of any shrimp hatchery can be achieved by integrating best management practices with environmentally sound prophylactic measures like probiotics in disease control and farming practices.

Guideline for selecting appropriate probiotics

The farmers choose the best one in terms of its performance in the field in general circumstances and ultimately the products which do not adhere to their claim simply vanished as it is rejected by them.

1. Understand the need for a probiotic application (Is it really necessary with regard to the system, stocking and species) and product profile and claim properly before selection for applying in the hatchery.

2. The method of application (culturing the microbes etc.) and the application strategy (dose and frequency) should be easy.

3. Views of the hatchery operators in and around with regard to that particular probionts can be obtained.

4. Before applying in the pond the GRAS (Generally regarded as safe) status of the probionts should be judged by a small field based experimental trial -should be non-pathogenic and non-toxic.
5. Capable of surviving and metabolising in the gut environment.
6. Ease, simplicity, practicability and minimum labor-intensive for its application.
7. Interaction with other food organisms like phytoplankton etc. can be observed.
8. It should not bring a drastic change in the abiotic or biotic factors.
9. Host challenged both in stressed and non-stressed condition and preferably in axenic condition for evaluation of the potential products.
10. Additional cost on production and return guaranteed in terms of quality /quantity.

Conclusion

Sustained production of healthy shrimp seeds are the major goals of any shrimp hatchery. Microbial interventions were initiated to make aquatic production more sustainable and disease management measures more environmental friendly. Maintenance of balanced populations of bacteria in a system is possible through the use of defined probiotics in a number of ways such as enrichment of larval food, inclusion in the diet, or addition to the water, as a remediation agent. It is evident that the manner in which probiotics impact as nutritional, immuno or environmental modulator varies depending on the specific strain, level present and environmental conditions. The best practices and the intrinsic and extrinsic factors to be considered depend on the nature of the microbial product and the mode of application and appropriate environment management. The mode of application could be broadcasting directly in water or through oral delivery. Probiotic induced immunomodulation especially the non-specific immunity and viral accommodation theory constitutively increasing specific immunity if at all, are to be explored to get better productivity and returns in the hatchery and in turn in the farming.

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Policies and regulations of Coastal Aquaculture Authority (CAA) for sustainable aquaculture development in India

Jayanthi, M

Shrimp aquaculture has grown very fast in the recent past in many tropical and subtropical countries, however there have been setbacks resulting from diseases, and the environmental and social impacts of shrimp farming. At the global level, rapid expansion of coastal aquaculture has resulted in large-scale removal of valuable coastal wetlands and subsequent loss of goods and services generated by natural resource systems. In India, aquaculture has transformed from a traditional to a commercial activity in the last three decades and the area under shrimp culture has increased manifolds. The rapid development of shrimp aquaculture in the coastal areas of the country also raised environmental issues such as conversion of mangroves, salinization of agricultural lands and drinking water resources and multi user conflicts. Issues raised over the unplanned aquaculture growth has made the sector under regulatory and licensing mode to control the indiscriminate growth, through the verdict by the Honorable Supreme Court of India. The Coastal Aquaculture Authority has brought out guidelines for the development of sustainable aquaculture (www.caa.gov.in) and is given below.

1. Guidelines for aquaculture as per CAA act 2005.

Coastal Aquaculture Authority Act was enacted in 2005 and a new Coastal Aquaculture Authority was instituted as per the Gazette Notification No. 1336 dated 22nd December 2005. The Aquaculture Authority constituted under the directives of the Supreme Court laid down certain conditions, related to the nature and conversion of the land used for shrimp farming, banning intensive and semi-intensive farming systems, requirement of Effluent Treatment Ponds and EIA etc., for issuing approval (license) for the shrimp farms. State level and District level committees were constituted by the State Governments for screening the applications on the basis of the above guidelines for recommendation to the Aquaculture Authority for issue of license.

- Under this Act coastal area for aquaculture includes the land within a distance of two kilometers from the High Tide Line of seas, rivers, creeks and backwaters.
- The delineating boundaries for coastal aquaculture along rivers, creeks and backwaters shall be governed by the distance unto which the tidal effects are experienced and where salinity concentration is not less than 5 ppt and ii. In the case of ecologically fragile areas, such as Chilka Lake and Pulicat Lake the distance would be up to 2 km from the boundary of the lakes.
- No license for aquaculture should be granted allowing aquaculture within 200 m of the high tide line or any area within the coastal regulation zone. However, this is subject to the provision that it does not apply to any aquaculture farm in existence at the time of the establishment of the Aquaculture Authority. Noncommercial and experimental aquaculture farms operated by any research institute of the Government or by the Government

- Mangroves, agricultural lands, saltpan lands, ecologically sensitive areas like sanctuaries, marine parks, etc., should not be used for shrimp farming.
- Shrimp farms should be located at least 100 m away from any human settlement in a village / hamlet of less than 500 population and beyond 300 m from any village / hamlet of over 500 population. For major towns and heritage areas it should be around 2 km.
- All shrimp farms should maintain 100 m distance from the nearest drinking water sources.
- The shrimp farms should not be located across natural drainage canals / flood drain.
- While using common property resources like creeks, canals, sea, etc., care should be taken that the farming activity does not interfere with any other traditional activity such as fishing, etc.
- Spacing between adjacent shrimp farms may be location specific. In smaller farms, at least 20 m distance between two adjacent farms should be maintained, particularly for allowing easy public access to the fish landing centers and other common facilities. Depending upon the size of the farms, a maximum of 100 - 150 m between two farms could be fixed. In case of better soil texture, the buffer zone for the estuarine based farms could be 20 -25 m. A gap having a width of 20 m for every 500 m distance in the case of sea based farms and a gap of 5 m width for every 300 m distance in the case of estuarine based farms could be provided for easy access.
- Larger farms should be set up in clusters with free access provided in between clusters.
- A minimum distance of 50-100 m shall be maintained between the nearest agricultural land (depending upon the soil condition), canal or any other water discharge / drainage source and the shrimp farm.
- Water spread area of a farm shall not exceed 60 per cent of the total area of the land. The rest 40 per cent could be used appropriately for other purposes. Plantation could be done wherever possible.
- Areas where already a large number of shrimp farms are located should be avoided. Fresh farms in such areas can be permitted only after studying the carrying / assimilation capacity of the receiving water body.

1.1 Shrimp farm registration and renewal

All persons carrying out aquaculture in the coastal areas shall register their farm with the CAA. Such registration made for a period of five years with facility for further renewal. Aquaculture will not be permitted within 200m from HTL and also in creeks, rivers, and backwaters with in the CRZ. However it is not applicable to the existing farms set up before CAA act 2005. Every application for the registration of a coastal aquaculture farm shall be made to the District Level Committee as set up by the Authority, obtainable from the office of the District Level Committee or the office of the Authority or be downloaded from the website of the Authority. On receipt of an application the District Level Committee shall verify the particulars given in the application in respect of all coastal aquaculture farms irrespective of their size; and

- In the case of coastal aquaculture farms up to 2.0 ha water spread area, the District Level Committee upon satisfaction of the information furnished therein shall recommend the application directly to the Authority for consideration of registration under intimation to the State Level Committee.
- In the case of coastal aquaculture farms above 2.0 ha water spread area, the District Level Committee shall inspect the concerned farm to ensure that the farm meets the norms specified in the guidelines with specific reference to the siting of coastal aquaculture farms and recommend such applications to the State Level Committee, which upon satisfaction shall further recommend the application to the Authority for consideration of registration.

The time frame of four weeks to the DLC for the detailed inspection and dispatching to SLC and two weeks for the SLC to give the recommendations are prescribed.

1.2 Regulations for SPF *P. vannamei* farms

- Aquaculture farmers who are registered with Coastal Aquaculture Authority will be required to submit a separate application for permission for farming *P. vannamei*. In case of so far unregistered farms, the application for registration must clearly spell out the intention to culture *P. vannamei*. Decision on such applications will be taken in accordance with these guidelines.
- Inspection team authorized by Coastal Aquaculture Authority shall inspect the farm and based on its recommendation regarding the suitability of the facility for farming of *P. vannamei*, applications shall be processed by the Member Secretary for consideration of the Coastal Aquaculture Authority for issuing permission to farms for farming of *P. vannamei.*
- Farms must establish adequate bio-security measures including fencing, reservoirs, bird-scare, separate implements for each of the ponds etc. The farms should be managed by the personnel who are trained and/or experienced in management of bio-security measures.
- *P.vannamei* shrimp is tolerant to low salinities but the rearing water should have a salinity of more than 0.5 ppt. The Govt. of India has notified that farmers who desired to culture vannamei outside the jurisdiction of CAA having the water salinity of above 0.5 ppt shall get registered with the Department of Fisheries (DoF) of the state government concerned. The farms should possess all the required infrastructure and biosecurity. The DoF may constitute a separate district level committee to inspect and give registration to the farms within a reasonable time frame of 60 days and other guidelines are same as that of brackishwater area.
- Farms irrespective of their size should have an Effluent Treatment System (ETS). Since loading of the environment with suspended solids is very high during the harvest, the ETS should be able to handle the waste water let off during harvest. Harvesting should be sequential depending on the size of the ETS. The quality of the waste water should conform to the Standards prescribed under the Guidelines issued by Coastal Aquaculture Authority.

2. Guidelines for culture of *P.vannamei* in fresh water / inland farms

Government of India have communicated approved guidelines that the farmers who desire to culture the exotic species, *P.vannamei* in fresh water/inland farms located outside the jurisdiction of the Coastal Aquaculture Authority(CAA), having a water salinity of 0.5ppt shall be required to register their farms with the State Fisheries Department. However, the farms located within the jurisdiction of the CAA shall register with CAA only.

The following guidelines and instructions for culture of *P.vannamei* in fresh water / inland farms located outside the jurisdiction of the Coastal Aquaculture Authority (CAA) having a water salinity of 0.5ppt:

- No person shall carry on the culture of *P.vannamei* in fresh water / inland waters without permission in accordance with this Order.
- The District Level Committee (DLC) constituted in the GO (2nd) read above shall be the Competent Authority to permit the culture of *P.vannamei* in fresh water / inland farms located outside the jurisdiction of CAA.
- Permission for taking up culture of *P.vannamei* shall be accorded only to farms which have been already registered with the Fisheries Department and which have complied with the guidelines.

2.1 Guidelines for granting permission for culture of *P.vannamei* in fresh water /inland waters:

- The DLC shall consider only the farms which are outside the jurisdiction of the CAA and water salinity in the farm is above 0.5 ppt.
- Permission shall be accorded within 60 days basing on the recommendations of the inspections conducted by the DLC members regarding the suitability of the farm for farming of *P.vannamei*.
- Stocking density should not exceed 60 number / sq. m.
- The farm should maintain a detailed record of the name and address of the hatchery from where the seed is procured, quantity of seed procured, water quality parameters and daily feeding data during the culture period in the prescribed format.
- Banned drugs and antibiotics should not be used (list is given in the CAA website)
- The farm must establish adequate bio-security measures including crab fencing, bird scare and separate implements for each of the ponds.
- If the farm is not connected to the outside water sources (rivers, canals, lakes etc.) the reservoirs need not be insisted for disinfection.
- The farms with connections to open fresh water sources like rivers or canals or lakes etc. which are geographically adjoining to brackish water areas, irrespective of their size should have an Effluent Treatment system (ETS). The quality of treated water should conform to the standards of the standards prescribed by the A.P. Pollution Control Board.
- In case of any outbreak of disease, the farmer shall report immediately to District Fisheries Officer. Distress harvesting is permitted through netting only and the discharge water should be chlorinated and dechlorinated before release into drainage systems.

- Farms approved for *P.vannamei* culture shall not be permitted for farming of any other crustacean species simultaneously.
- Tested and certified seed should be procured only from the hatcheries approved by the CAA for *P.vannamei* seed production.
- For ponds not connected with open water sources, the accumulated organic wastes should be removed and disposed of safely.
- Farms located within the jurisdiction of CAA shall register with CAA invariably.

2.3 Advisories for sustainable culture of SPF *P.vannamei* in fresh water/ inland farms.

- It is advisable not to culture in fresh water with 0 ppt salinity since it could lead to poor growth, poor survival and poor quality
- Lower stocking rate is advised to reduce the operation cost and improve sustainability.
- Gradual acclimatization of the post larvae to the existing salinity conditions is very important for ensuring good survival.
- Younger stages of larvae below 15 days age old will not be able to tolerate lower salinities, hence PL15 and above should be used. In case of inland saline water culture, the ionic composition of pond water should be assessed continuously with respect to Potassium, Magnesium and Calcium for making necessary amendments.
- Feed with proper fortification of minerals as required should be followed for ensuring better survival rate and growth.
- Only probiotics suitable to the culture environment should be used.

3. Conclusion

Sustainable coastal aquaculture hinges on environmental protection and social responsibility. The guidelines are framed to ensure environment friendly, socially acceptable and sustainable aquaculture which should not disturb the other production systems and end users of natural resources. As self-discipline is the secret of sustainability, the shrimp farmers and other stakeholders need to follow the regulatory guidelines and should integrate themselves with the Coastal Zone Development programmes so that shrimp farming can be sustained and continue to help in improving the socio-economic capabilities of the coastal population.

Pond preparation and prestocking management measures in grow out shrimp production

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The success of any shrimp culture depends on the better management practices involved in pond preparation and pre-stocking management steps. Pond preparation is one of the most important pre-stocking management measures essential for optimum growth of shrimp in growout farming systems. There are various points to be taken care of during the pond preparation for shrimp culture.

Drying the pound bottom

After each harvesting cycle, the pond bottom is allowed to dry and crack. It helps to oxidize the decomposed organic components, leftover in the pond after the previous culture. Generally, pond bottom is allowed to dry for 7-10 days and, it allows soil crack to a depth of 25-50 mm. It helps to reduce the risk of disease outbreaks and improve shrimp production.

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. Tiling and ploughing are not generally recommended in acidic soils as it increases the soil pH.

Top soil removal:

The top black soil and bottom sludge to be removed to prevent the development of anaerobic condition during the culture period. The sludge must be disposed of away from the pond site so that it does not seep back into ponds. Grow out the 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.

Top soil removal

Pond bottom after top soil removal

In the case of undrainable ponds, water must be pumped out through submersible pump and repeated flushing of water be done followed by quick lime application 100-200 kg per ha to disinfect the heavy organic load. Areas with acid sulfate soils are not unadvisable for prolonged sundry as it can lead to oxidation of iron into its oxide which can later in contact with water result in the sulphuric acid formation and reduction in soil Ph. Potential acid sulfate soils (PASS) are pyrite-bearing sediments that have the potential to oxidize and generate sulfuric acid when exposed to oxygen. Although PASS is usually associated with mangroves and other intertidal settings, many brackishwater ponds in coastal states such as Kerala proximate to mangrove system are affected to have acid sulfate soils.the These type ponds are not suitable for complete draining. Dykes constructed from PASS will oxidize and supply acid by direct contact with pond water and run-off from the dike Lime bags provision or application of lime over soil dike helps to increase the ph level in acid sulfate soils.

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 the 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 >5 and Quick lime or hydrated lime can be applied if soil pH below 5. Where disinfectants like bleaching powder (calcium hypochlorite) are used, 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

Table 1. Amount of lime (tons/ha) to raise the soil pH to 7.0.

Soil pH	Quantity of lime material (tons/ha)		
	Dolomite	**Agricultural**	**Quick lime**
6 to 6.5	5.7 to 2.8	5.5 to 2.8	4.6 to 2.3
5.5 to 6.0	8.5 to 5.7	8.3 to 5.5	6.9 to 4.6
5.0 to 5.5	11.3 to 8.5	11.1 to 8.3	9.2 to 6.9
4.5 to 5.0	14.2 to 11.3	13.9 to 11.1	11.5 to 9.2
4.0 to 4.5	17.0 to 14.2	16.6 to 13.9	13.8to 11.5

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. Generally, bleaching powder @ 20-60 ppm is recommended for reducing a load of harmful bacteria and virus in the cultured water. Optimum water quality criteria for intake water are given in Table 2. Keeping a suitable reservoir also facilitate chemical treatment to reduce disease outbreak and to make water management more effective during the production cycle.

Table. 2. Optimum water quality criteria for intake water

	Parameters	Range
1.	Temperature (^{0}C)	25-33
2.	Salinity (ppt)	10-34
3.	pH	7-9
4.	Transparency (cm)	25-50
5.	Dissolved Oxygen (ppm)	4-6
6.	Total Alkalinity (ppm)	50-300
7.	Nitrate- N (ppm)	< 0.03
8.	Nitrite- N (ppm)	< 0.01
9.	Ammonia- N (ppm)	< 0.01
10.	Heavy Metals (ppm)	Nil to 0.0001

Fertilization of pond water

The purpose of fertilization is to ensure the growth of primary producers in culture ponds. They initiate a natural food web in the aquatic ecosystem and directly or indirectly contribute to shrimp growth also. It also helps to maintain a desirable level of transparency (25-40 cm), which prevents the development of harmful benthic algae. Phytoplankton in culture ponds also helps to improve the water quality parameters in grow-out ponds.

Fertilizers can be applied depending on the fertility status of the soil. Organic fertilizer like dry cow dung at the rate of 500 – 2000 kg ha^{-1} and inorganic fertilizers like urea and single superphosphate (SSP) at 25 – 100 kg ha-1 can be applied depending on the organic carbon content (1.5-2.5%) , available N (50-75 mg/100 g soil) and available 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. The brownish green color of pond water indicates that the pond is ready for stocking the seed.

Application of organic-based indigenous probiotic

Yeast-based organic preparation is recently being used in zero water exchange shrimp culture ponds as a probiotic. It can be prepared using ingredients like 60 kg paddy flour, 30 kg molasses and 2-4 kg yeast *Saccharomyces cerevisiae*. Allow these ingredients to get ferment in 48 h and can be applied in one ha pond. Organic juice can be applied in a biweekly interval to improve the fertility status of water @ 2 ppm.

Seed Selection and Stocking

Stocking of shrimp is one of the most important components of a biosecurity program. Use seeds produced from domesticated shrimp stocks that are free of specific diseases (“Specific Pathogen Free” or SPF) and or with stocks resistant to specific disease agents (SPR) SPF broodstock from certified Nucleus Breeding Center (NBC). These are biosecurity facilities where there are two or more years of documented disease testing to support their SPF status. 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 stock, the entire batch should be rejected. Before stocking at the pond, PL should be treated with formalin at 100 ppm concentration for 30 minutes inwell-aerated tanks to remove weak PL. Maintain a balanced or optimum stocking density is also an important component of shrimp culture

Pond bottom and water quality management

Disease outbreaks in shrimp grow out culture are directly related to the pond bottom and water quality. Zero water exchange must be followed throughout the culture period to prevent disease occurrence. Use chemical like KmNo4 dip treatment may be followed to disinfect all equipment– screen net, cast net, trays or accessories while sampling and regular monitoring (surveillance) of shrimp stocks. In case if soil redox potential ranges beyond -200 mEv, soil amendment procedures like soil lime and KmNo4 mix application after 6 pm followed by lime application in the morning can be continued. Chain dragging also helps to release escape of obnoxious gas and organic load oxidation in the pond bottom.

Better-Feed management

Cost of feed accounts for about 40% to 60% of the total production cost. Do not use fresh feed, trash fish, bivalves, etc. as it can carry vectors. Feed monitoring should be done with the check tray evaluation for optimum feed management. Feeding area can be shifted at least once in 7 to 10 days, depending on the bottom condition along the feeding area. Reduce feeding during periods of low DO, plankton crash, rain fall, extremes of temperature, etc. Slightly under feeding is better than over feeding, which saves money and reduces disease risks and during disease outbreaks. Proper storage of feed is also an important component in the biosecurity plan.

Shrimp Health Monitoring

Shrimps should be sampled once in a week by cast netting and should be checked for their general health conditions like external appearance, For example; a pale, whitish gut showed gut infection while a normal gut will have a light or golden brown color. Probiotics, immunostimulants, bioremediating agents can be employed as prophylactic measures in grow out culture. Yeast based organic preparation(60 kg rice flour, 30 kg yeast, and 3 kg yeast) application can be applied to improve the overall pond microbial balance. Since there is a serious concern on the use of antibiotics, their use in shrimp farming should be avoided.

Farm Record Maintenance

Records are necessary to identify various risks 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. Control workers' movement in and across the farm and minimize the number of workers in stocking, harvest, sampling, etc. It is utmost important to make environmental cleanliness and control human traffics – guest, workers, technicians, and movement across the farms.

Better Practices for harvesting

Harvesting must be avoided during the moulting period, and agricultural lime can be applied 3-4 days before harvesting. Try to do harvesting in the early morning or evening with dragneting with minimum delay. After harvesting transport crates with crushed ice at a 1:1 ratio for better preservation. Nutrient-rich pond effluent must be treated before get discharged to the water source.10% of the pond area are advised to be kept for effluent treatment pond before the discharge of cultured water in to open water bodies.

Engineering perspectives in pond design and operations including alternative energy-based system

Nila Rekha, P

Introduction

The vast majority of shrimp culture in the world is conducted in outdoor earthen ponds that are typically located in coastal zones. The pond design and construction in conjunction with the cluster of shrimp farming at a particular place needs to be suitable and site-specific. A shrimp farm should be designed according to the characteristics of the selected site and the culture system. There is no unique design, but optimum and functional farm layout plan and design should be based on the physical and economic conditions prevailing in the locality. The ponds and buildings should be laid out for efficient and economic operation and the best utilization of the land. Construction of ponds and drainage systems should be planned and supervised by both an aquaculturist and an engineer, particularly if a large system is to be constructed.

An ideal shrimp farm is a complex establishment consisting of

(a) various size ponds for nursery and grow-out,

(b) water control structures including embankments, supply and drainage canals and sluice gates

(c) support facilities such as roads, bridges, living quarters, workshops and warehouses, etc.

Careful layout of the described facilities and appropriate structural design in relation to the physical features of the area ensure smooth and effective operational management. The improved structural design is largely required due to the behavior of the Penaeid shrimps. General guidelines and protocols to be followed in designing has been given below

Topography and type of pond

The topography of the land will in part determine the type and shape of some or all of the ponds. There are three basic structural types of ponds.

- Excavated pond
- Levee ponds
- Ravine ponds

The most common type is the excavated pond in which earth is removed and used for building the banks. This type of pond can be constructed on flat or undulating land. The construction has been done by hiring a tractor and forming the bund at cheaper cost. Levee ponds are constructed on very flat land and are similar in structure to rice bays except that the banks must be high enough to contain the necessary depth of water. Gully or ravine ponds are restricted to hilly country and are constructed by damming valleys or gullies.

Pond design

Earthen ponds comprise the major capital investment in aquaculture facilities throughout the world. More than 90 per cent of the total global production is from pond culture.

A well-designed pond will facilitate the management of water exchange, harvesting of the product, waste collection and elimination, and feeding. It would allow circulation of the water such that wastes will be accumulated at the center of the pond.

Size and shape of culture ponds

Ponds should be square or rectangular to make the most efficient use of available land. It is more economical to construct square ponds; however, rectangular ponds are easier to manage. The longest axis of a pond should be parallel to the prevailing wind direction. This facilitates water movement generated by wind action thereby increasing dissolved oxygen in the water and minimizing water temperature fluctuations in summer or warmer months. The breadth of a pond depends largely on the purpose and the operational system employed. Some farmers improve the water movement in the square and rectangular ponds by making the corners of the pond rounded through addition of soil. The following are the various sizes recommended for shrimp culture practice:

Table 1 : Pond size for different culture system

Type of pond	Type of culture system	Size of the pond
Nursery pond		500 to 1,000 m^2
Grow-out pond	- intensive	0.25 to 1.0 ha
	- semi-intensive	0.5 to 2.0 ha
	- extensive	1.0 to 10 ha

Smaller ponds are easier to manage but the construction and operation can be costly. Ponds of 0.5-1.0 ha. are commonly used in intensive culture and 1-2 ha. for semi-intensive culture. There is a large variation in the size of earthen ponds used in aquaculture throughout the world and authorities disagree on the optimum size of pondsA number of factors will determine the preferred size of ponds on each farm: the function, techniques and stocking densities, cost of land, topography, capital and equipment available for construction and the planned production capacity. Construct ponds no larger than about 2 ha to enable the efficient management necessary under intensive conditions.

Dikes

Dikes do not only serve as boundaries to indicate pond size and shape but also function to hold water within the pond as well as protecting other farm facilities from flood. Diking materials must preferably be tested for load bearing capabilities and compactibility. In some cases where the quality of the soil is inferior for diking, other materials, viz: concrete or clay must be used as core materials to be placed at the pond bottom. Earthen dikes, with or without lining, are found to be the most economical. Design and construction of embankment must be based on sound engineering principles and economic feasibility.

Height of dike: Coastal soil used as diking material usually shrinks initially. As such, the height of perimeter dike should have a free board of 0.6–0.7 meter above the desired water depth. To compute for the height of dike, the following formula could be used:

$$H = \frac{(Hw - G) + FB}{1 - \%\ \text{of shrinkage}}$$

H = height of designed dike

Hw = highest high water level from past record

G = ground level over mean sea level

FB = height of free board

% = percent shrinkage

To give a concrete example, let us assume that a proposed shrimp farm has a ground elevation of 1.0 meter above mean sea level and normal high tide of 2 meters. Previous records indicate that the highest tide occuring every 10 years is 2.8 meters. The rate of soil shrinkage after the embankment have been consolidated is 20% and the estimated free board allowed is 0.60 meter. Height of dike is then calculated from the formula:

$$H = \frac{(2.8 - 1) + 0.6}{1 - 20\%}$$

H = 3 meters

Dyke Slope: The slope of perimeter dike is maintained at an average ratio of 2:1 to 3:1. Very often, external slopes are made at a ratio of 2.5:1 to 3:1. Dikes with steep slopes are always subjected to erosion and require higher maintenance cost Slope of a dike also highly depends on soil quality.

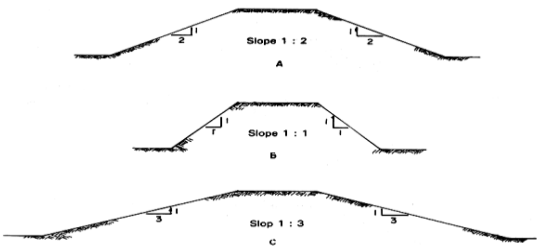

Fig 1. Dyke slope

Dikes should be designed to impound higher than 1 m. depth of water and must be high enough to prevent flooding during the rainy seasons and the highest high tide. The slope of the dike depends on the nature of the soil. A slope of not less than 1:1.5 is normally used in the sandy soil area to avoid erosion and 1:1 is used for clay soils. One must be aware that shallow slopes will encourage the growth of benthic algae which will impair the quality of the water in the pond. Some dikes in a farm may be wider than the others to provide space for the access road, storage, electricity and aerators.

Pond bottom slope: Pond bottom should be as even as possible; free from projecting rocks and tree stumps. The bottom must have a gradual slope from the inlet gate towards the drainage gate. The suggested ratio of the slope is 1:500.

Pond Lining

Lining materials are used in pond where the soil contains a high percentage of sand, and organic matter and is acidic in nature. Lining can reduce erosion, water seepage, waste accumulation in the soil and the leaching of ammonia, hydrogen sulfide, acidic compounds, iron and other potentially stressful compounds into the ponds. The lining also allows easy removal of wastes from the feeding areas, reducing the time and costs to clean the ponds between cycles. Several lining materials are currently available, including the compact laterite, compact clay, bitumen impregnated polypropylene textiles (geotextiles), polyvinylchloride (PVC), polyethylene (PE) and high-density polyethylene (HDPE). Farmers may line the pond totally or partially, depending on economic/financial consideration. Another factor will be the rate of waste accumulation in an area on the pond bottom. The economic life of liners varies according to the maintenance and the duration of exposure to sunlight.

Experimental studies were conducted in CIBA experimental farms on various cost effective seepage control structures.

Pond depth

The rearing pond must have a minimum depth of 1.0 meter. Most traditional brackish water ponds for shrimp farming are relatively shallow. To satisfy depth requirement, a ditch is constructed along the dike or a central canal between two opposite sides of the pond. The average depth is 1.0 – 1.2 meters and depth of the platform is 30–60 cm. Such pond design with peripheral ditches and central platform affords several benefits:

a. The ditch provide better living conditions during hot weather.
b. The shallow, centrally located platform serves as growing area for the natural food organisms.

The ditch also serves as harvesting canal

Water Supply System

A shrimp pond is filled with water mostly by pumping. The pumps should be installed at locations where they can obtain water from the middle of the water column with least sedimentation and pollution. The pumps and inlet canal should be large enough to allow the ponds or the reservoir to be filled within 4-6 hours. A screen should be installed at the inlet canal prior to the pumps to prevent clogging at the inlets. Influent water system is very important with the biosecurity perspective.

Reservoir pond

A reservoir is important for the control of pond environment and storage of water supply when the water quality is inconsistent or the supply is intermittent. It is recommended that the area of a reservoir within a farm should be about 30% of the total farm area in order to hold a sufficient volume of the water supply.

Design of canal

Not all shrimp farms are located close to the coast or estuaries. For those that are located far away from the water sources, it is necessary to construct supply and drainage canals.

Conceptually, a shrimp pond must possess separate canals for drainage and supply and for avoiding probable contamination of the water supply. Both supply and drainage canals would likewise serve as water level control in the pond and as temporary holding areas for shrimps. It is important that the siting of the canal systems takes advantage of the natural waterways within the proposed site.

Dimensions of supply and drainage canals are calculated by using the following equation:

$Q = AV$ Where:

Q	=	volume of water discharge
A	=	cross-sectional area of the canal
V	=	velocity of water flow

V value can be calculated by the following formula:

$V = R^{2/3} \times S^{1/2} \times 1/n$ where:

R = depth of water flow
S = canal bed gradient
n = coefficient of roughness (0.02)

Each pond should have a separate inlet and outlet. Both should be screened; the inlet to prevent the entry of trash fish and other undesirable aquatic fauna, and the outlet to prevent the loss of stocked fish. The diameter of supply and drainage pipes should be at least 15 cm. Lay all pipes underground and do not plant trees close to drainage or supply lines. Construct ponds so that they can be drained individually, completely and rapidly. This will enable the removal of all fish during harvesting and facilitate efficient management, particularly when water quality and disease problems occur. Complete drainage can be achieved by a raceway or well in the deeper section of the pond. The bottom of the pond should be level and slope gradually towards this area. The outlet structure should enable the adjustment of water level and also allow for the overflow of excess water. It is important that water can be drained from the bottom as well as the surface, so that the 'dead' water (low or deficient in oxygen) can be removed.

Gates for Inlet and Outlet

Each shrimp pond should have at least one gate for filling and draining water. However, a typical pond of 0.5-1 ha. usually consists of two gates having similar structure for the inlet and outlet gates. The size of the gate is dependent on the size of the pond, but must allow the pond to be filled or drained within 4-6 hours. Gates of 0.5-1.0 m. wide are usually constructed, since gates wider than 1 m. will cause difficulty in screening and will allow strong currents which will cause erosion of the soil. The position of the outlet should be at the lowest point of the pond with a gradual slope of 1:200 from the inlet to allow total drainage of the pond during harvesting.

The conventional gates constructed at the side of the pond should have a double screen, with fine a mesh for the initial period of culture and a coarser one for a later period. Some farmers may place both meshes in a single frame and cut out the finer mesh when the size of the shrimps are larger than the opening of the coarser mesh.

Central Drain

This has been employed in some farms and consists of perforated pipes laid horizontally at the center of the pond and connected to a pipe leading to the outlet. A screen of small mesh size is used to cover the drain for the first 50 days of culture and is removed to allow for easy removal of water when the shrimps are larger than the diameter of the pipe. This method has the advantages in that it can remove the waste and clean the pond bottom any time throughout the culture period.

Several techniques to accomplish carrier exclusion will lead to record production. First they minimize water exchange in what they refer to as the closed or zero-exchange system in which the pond is filled upon stocking and then maintained without replacement for the entire culture cycle of 120- 130 days. Some farmers cautiously compensate for evaporation and infiltration losses toward the last half of the cycle, but all of them strive to minimize exposure to carriers by very discriminating exchange of water. Note that this 'closed' approach is highly dependent on mechanical to maintain satisfactory water quality for the duration of the crop cycle.

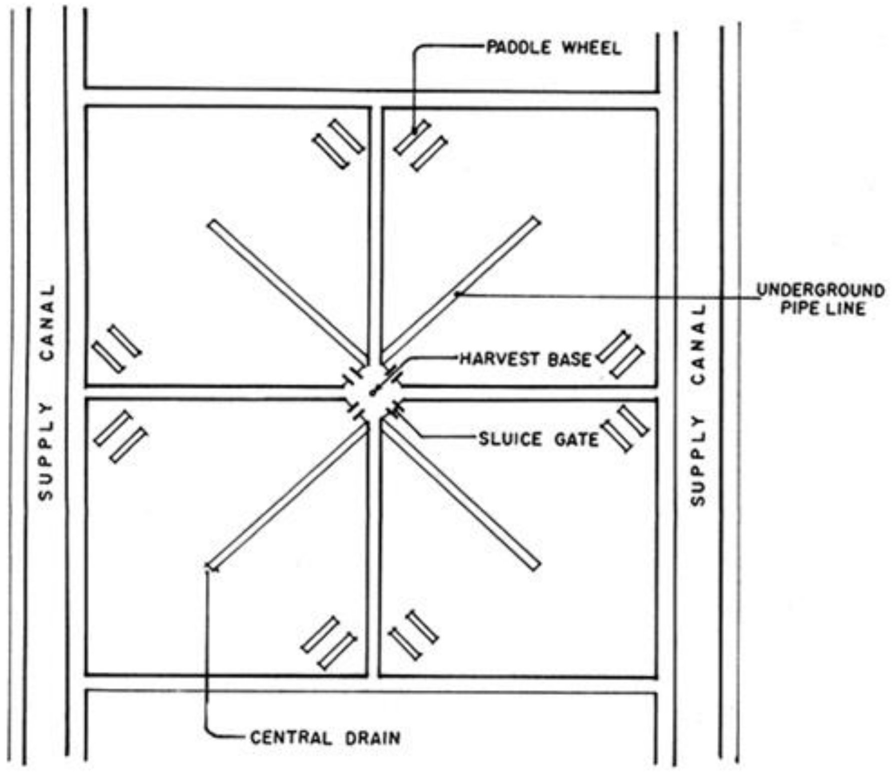

Fig 3 Typical intensive pond with central drain

Drainage Canal and Sedimentation pond

The drainage canal of a shrimp pond should be at least 50 cm. lower than the lowest point of the pond to allow drainage by gravity. The effluent will be drained into a sedimentation pond to settle the particulate wastes before water is pumped into the reservoir or released out of the farm. It is recommended that the sedimentation pond should be approximately 5-10% of the culture area and should be deep enough to prevent mixing and re-suspension of the wastes. Baffles or soft walls made of fine mesh net or plastic sheeting supported by stakes driven into the pond bottom, may be constructed in the sedimentation pond to decrease the velocity of water and increase the retention time which will enhance the settlement of the wastes. The wastes in the sedimentation pond should be removed periodically and discharged into the waste dumping area.

Waste Dumping Area and buildings

A shrimp farm should provide 5-10% of the area for dumping of the wastes. Wastes from the pond must be collected carefully and dumped into this area without discharging to nearby areas, which will contaminate the natural resources

Accommodation, storage, shop and guard houses may be built in the farm as required. It is advised that accommodation for workers should be set up at various points around the farm for security purposes and to allow the ponds to be adequately monitored.

Renewable energy sources

Renewable energy sources also called non-conventional energy, are sources that are continuously replenished by natural processes and can never be exhausted**.** While most renewable energy projects and production are large-scale, renewable energy technologies are also suited to small off-grid applications, sometimes in rural and remote areas, where energy is often crucial in human development. The aquaculture sector can be energised with all the above forms of renewable energy

Aquaculture sector - energy requirement and consumption

The biological, technical and commercial feasibility of aquaculture enterprises are often related to local environmental conditions and an available niche market potential. With regard to its direct economic potential it involves information about fixed and running costs with special focus on the operation and production costs.. With greater intensity of production, dependence on external resources expands and financial performance is governed to a large extent by input availability and costs, including that of energy. Production in intensively managed systems in particular is vulnerable to fluctuating energy prices and interruptions in supply. Direct energy consumption in intensively managed aquatic farming systems, notably for shrimp where the energy requirement for aeration is huge. However, in some states the subsidised fuel costs for agricultural and aquaculture use, mean farmers are to some extent protected.

Similarly, for Recirculatory aquaculture system has by far the greatest energy demand and usage. Higher direct energy consumption in shrimp farming as compared with cage farming energy consumption is associated with: site development and construction; production, acquisition and supply of inputs; waste handling and disposal; product processing, marketing and distribution. Extensive aquaculture operations require little in the way of site development and construction; semi-intensive aquaculture systems including earthen ponds, wood and bamboo pens and cages and cove culture account for modest energy consumption in preparing the site and construction.

Solar energy

Aquaculture activities being carried out in remote area, this technology has wide spread application viz.

- Solar pump for water pumping
- Solar powered automatic feeder for dispensing shrimp and fish feed in ponds
- Solar powered water quality monitoring gadget
- Solar drier for drying shrimp and fish feeds

In general, solar power requires a high one-time capital investment, with relatively small operating costs for a solar plant life of 25 years. One drawback of solar PV that can be minimized over a period of time pertains to the cost of solar panels which is expected to drop. Solar PV-based captive power production is likely to provide significant benefits when compared to diesel-based power, especially for back-up power. The future of captive power generation through solar PV is bright as industrial demand will continue to increase, and activities like trading through private exchanges will make it easier for captive power generators to sell the surplus for cash benefits

Back-up systems are necessary since PV systems only generate electricity when the sun is shining. The two most common methods of backing up solar electric systems are connecting the system to the utility grid or storing excess electricity in batteries for use at night or on cloudy days.

In CIBA we have designed and developed solar powered timer controlled automatic feeder for *P. vannamei* pond for a capacity of 125 kg.

Greenhouse

Employing a greenhouse reduced conventional energy requirements in hot dry climates and cooler temperate climates by 87% and 66%, respectively.

Bio energy for Aquaculture

Fish production integrated with renewable energy derived from waste-to-energy plant: including integration with intensive irrigation (in greenhouses, in cold climates), and based on:

- The Energy center providing electricity and waste heat to enable year-round production of fish under any climatic conditions (in closed systems).
- The Fish production system discharges sludge, and the Fish Processing Plant provides scrap, both converted to Liquid Fertilizer by the bio-digester, to be used by the greenhouse to improve vegetable production, or to produce biodiesel.

A feeding management system for aquaculture – Hybrid of solar and wind

Minimizing fish-feed loss requires significant monitoring that must be set-up to collect relevant information on-site. As a result, substantial energy requirements are needed in a cost-effective way. Moreover, operational issues must also be tackled toensure that core operations of the fish farm remain uninterrupted. Renewable energy sources (RES) might have the answer. Accordingly a hybrid system consists of Polycrystalline PV panels, a small wind generator, a charge controller to protect the battery bank, and the energy storage system (batteries). The system operates at 12V DC

Conclusion

Aquaculture production systems could be successful if appropriate site-specific farm design was conceived and constructed with the coordination of an engineer and biologist. By following internationally agreed upon policies and guidelines and a variety of management strategies successful aquaculture could take place.

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Mud crab grow out culture

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Mud crabs are one of the most traded aquaculture crops. The pond based farming of mud crab has been practicing in China for at least 100 years and more than 30 years in Asian countries (Balalio 2004). However, the mud crab was an incidental or secondary crop in shrimp and milk fish farming until recently. Owing to the high market value and profitability, the aquaculture of mud crab has received impetus in early 1990s. Considerable efforts have been made in the last few years to develop effective grow out technology for mud crab. Any aquaculture industry is composed of three sequential phases: seed production, nursery and grow out. This lecture note covers various aspects of grow out procedure of mud crabs. Pond based aquaculture system of mud crab comprises two approaches: 1) rearing of juvenile crabs until marketable size and 2) fattening of recently molted crabs (Fig 1).

Grow out: Rearing of juvenile crabs

Farm design: Rectangular ponds with a size ranging from 250 m^2 to 10,000 m^2 (1 ha) area is suitable for mud crab pond construction. Essentially, any shrimp farm cab be modified into mud crab farm. Although mud crabs are found to be tolerate wide range of salinity from 0 ppt to 40 ppt, salinity above 34 ppt and below 10 ppt are found to be less suitable for pond culture. If there is a probability to enhance salinity above the optimum level in summer months, it is recommended to reduce the salinity by diluting with fresh water (Balalio 2005). However according to regulations of Coastal Aquaculture Authority rules it is not acceptable.

The crab ponds should have a minimum water depth of 1 m and further, each pond should have ~12 earthen mounts (~ 5 m3). The top surface of these mounts should be above the water surface (Fig 2). These mounts are breathing space for crabs when dissolved oxygen level of ponds drops below the optimum level. The ponds must be fenced with nylon netting to prevent the escape of crabs, and it should be extending minimum 50 cm above the water line. Further, a strip of plastic should be installed over the fence (about 30 cm width, Fig 3). The lower side of the netting is embedded 10 cm below the base of enclosure.

Pond preparation: Pond preparation strategies generally employed in shrimp/ prawn aquaculture can also be adopted in mud crab aquaculture. However, it is generally believed that meticulous and stringent pond preparation is not required. The installations like net fencing, earthen mounts should be considered. Pond should be drained and keep it for 1 week. If it is not drainable pond, the pest should be eradicated by applying tea seed cake or powder (15 to 30 ppm).

The procedure adopted by farmers for pond preparation is not available as in the case of shrimp aquaculture. Here we provide a protocol used by SEAFDEC researchers in their experimental culture (Trino et al 2004). It can be modified according to the site and location of the farm. Liming and fertilization is the best way to increase the natural productivity of pond. Liming enhance the general health of the pond ecosystem. There are several types of liming material, and most common being agricultural lime stone, burnt lime and hydrated lime. Of these agricultural lime is found to be best, and it can be applied at the rate of 1 mt per ha. Inorganic fertilizers are applied to increase the phytoplankton productivity in shrimp aquaculture ponds; however, the utility of fertilization in crab aquaculture is not evaluated. It is however essential when crab aquaculture is integrated with seaweed culture. Fertilization with urea at the rate of 25 kg/ha and ammonium phosphate at the rate of 50 kg/ha is recommended.

Transportation and stocking: Farmers of mud crab rely on small crabs or juveniles (25-50 g) sourced from inter tidal flats, estuaries and mangrove to stock grow-out ponds. Handling, packing and transport activities are stress to animals. Nevertheless, crab juveniles are relatively easy to transport by using cane basket, carton lined with moist sea weeds or mangrove leaves (Fig 3). Chelae are tied to prevent fighting among crabs. In air, mud crabs have a life span of 2-18 days when packed with moist marine algae, cotton or wood shavings (Vasudeo, RB and Kewalramani, HC. 1960. Transport of the common crab *Scylla serrata* in living condition. Indian J. Fish. 7: 169-173). Stocking should be done with seeds having intact appendages, and without injury, and further seeds should be at uniform size. Differential size leads to cannibalism. Seeds should be stoked when water temperature is low; early morning or late evening preferably night. Stocking density in mud crab culture is generally far less than the shrimp farming. The stocking density has a major effect on crab growth, survival and production, and it is generally ranged between 0.5 and 3 crabs/ m^2. Several experiments were carried out to assess the optimum stocking density in mud crab aquaculture. Trino et al (1999) from Philippines compared the effect of three levels of stocking density (0.5, 1.5, and 3. 0 crabs/ m^2) on the growth performance of mixed species of mud crabs, *Scylla serrata* and *Scylla tranquebarica* (larger forms). Although there was no significant difference in the growth rate among different stocking density groups, highest harvest size, survival and efficient FCR were significantly higher at the lowest stocking density, and they concluded that mud crab culture at 0.5 and 1.5 crabs/ m^2 is economically viable.

Nutrition and feeding

Despite the growing interest of mud crab aquaculture, formulated diets for grow-out mud crabs have yet to be available, although research institutes like CIBA and CMFRI are at the various stages of commercialization of formulated crab feed. Management of feed is the most crucial element for successful aquaculture as feed is the major input of crustacean aquaculture. Feed accounts for 40 - 50% total operating cost (Trino et al 1999).

Natural diet of mud crab mainly includes crustacea and mollusks, whereas fin fish remnants are found to be very scarce. This is mainly due to the inefficiency of crabs to prey upon the fast moving preys. In the grow out culture management, locally available cheap protein sources (trash fish, mollusks) at the rate of 8-10% of biomass can be given. The crabs can be fed a mixed diet of 25% fish bycatch (trash fish) and 75% fresh flesh of mollusca or crustacea. Crab biomass can be estimated as the product of mean body weight of stocks in the enclosure and percentage survival. Linear decrease of 5% at every 15 days can be used as an assumed survival (Rodriguez et al 2003). An example for feed calculation is given in the

Table (1). Rodriguez et al (2003) further report that better growth for mud crabs obtained when fed with molluscan meat than trash fish, although results are not significant. While comparing the production performance of mud crabs using three different feed treatments, crustaceans, trash fish and without feed, Christensen et al (2004) found no significant difference among the treatments. They concluded that endogenous biota of culture system contributes a significant level of nutrition to crab as their data does not show any significant difference fed and unfed pond ponds. They also assumed that feed input may deteriorate the pond conditions of fed pond and it may be the reason for low survival of crabs in these ponds.

Feeding rate in % wet body weight (BW) used throughout the experiment and estimated survival (%) from stocking

	0-30 days	*30 – 60 days*	*60-80 days*	*Above 90 days*
Feeding rate (%)	15	10	7	5
Estimated survival (%)	100	100	70	70

Water quality characteristics: The water depth should be maintained at 80-100 cm level. The water should be replenished regularly, Rodrigueiz et al 92003) and Trino et al (1999) suggest that water should be exchanged three consecutive days during the spring tide. Generally water should be refreshed at the rate of 40% during the first months, 50% during the second month and 60% during the third month. Water quality characteristics should be monitored regularly. The acceptable optimum level of water quality characteristics are given in the Table 2. If water quality remains within the optimum level, the water exchange is not required.

Table 2. The acceptable optimum water quality levels in mud crab grow out ponds

Variables	Range
Temperature (^oC)	23 – 33
Transparency (cm)	25 – 45
pH	7.5 – 8.5
Dissolved oxygen (ppm)	>3
Salinity (ppt)	10 – 35
Total alkalinity (ppm)	200
Dissolved inorganic phosphate	0.1 – 0.2
Nitrate – N (ppm)	<0.03
Nitrite – N (ppm)	<0.01
Ammonia – N (ppm)	<0.01
Cadmium (ppm)	<0.01
Chromium (ppm)	<0.1
Copper (ppm)	<0.025
Lead (ppm)	<0.1
Mercury (ppm)	<0.0001
Zinc (ppm)	<0.1

Harvest and post harvest: Culture period is generally 3 to 6 months and is determined mainly by the size at stocking and the preference and demand, existing in the market. Culture period may be restricted to 60 days, if the crabs having a size of about 250 gm are preferred in the market. Culture duration will be 150 days for *S. tranquebarica* from an initial size of 25 g to a harvestable size of 350 – 450 g, if the stocking density is 1 crab per m^2. To obtain a harvestable size of 800-1000 g the culture has to be extended further up to 7 months. For *Scylla serrata*, culture duration will be 120 days with an initial size of 25 g and harvestable size of 200-300 g if the stocking density will be 1 crab per m^2 . To obtain larger sizes (400-500 g), culture period can be extended to further 3 months. Harvest of crabs can be effectively done in a tide-fed pond by letting in water through the sluice gate into the pond during incoming tide. As the water flushes in, mud crabs tend to swim against the incoming water and congregate near the sluice gate from where they can be caught with the help of a scoop net. Partial harvest can be made with baited lift nets and bamboo cages/traps. To have a total and complete harvest, crabs are to be hand-picked after completely draining the culture pond. Crabs should be tied immediately after their capture in order to curb their movement and to avoid the fighting among themselves and thereby losing their legs. Tying is a process in which a nylon/jute thread is placed in between the frontal portion of the body and the chelipeds and is coiled around their fingers after keeping the chelipeds in folding posture and subsequently both ends of the thread is put into a double knot at the rear end of the crab. the “water crabs” encountered in the final harvest can be utilized for fattening purpose. The tied-up crabs are to be initially washed with fresh sea water and subsequently sent for local marketing after packing them in bamboo baskets, in which, they are kept in layers alternatively with materials such as wet seaweeds or moist wood shavings or cotton soaked with sea water to keep the crabs in cool and moist condition. Those crabs exported in live condition, are given a fresh sea water dip and packed in perforated thermocole boxes for air shipment. The expected survival rate during culture would be around 70 to 80 %. Mud crabs are generally sold in live condition for both local consumption and live crab export trade. For the purpose of marketing, the mud crabs are graded as “extra large” (1 kg and above), “large” (500 g to less than 1 kg), “medium” (300 g to less than 500 g) and “small” (200 g to less than 300 g). The female crabs with fully developed ovary are usually sold for a higher price. Live and meaty mud crabs weighing above 300 g are considered for export, while the undersized live crabs (less than 300 g) and those live crabs which have lost their legs are sold in local markets. While marketing, about 20 % mortality is observed when the transport is by sea whereas transport by air reduces the mortality to about 5 to 10 %. Packing in ventilated and insulated containers instead of cardboard boxes, with 95 % relative humidity and 16 – 20^0 C temperature, will reduce the mortality of the mud crabs during transit up to 7 days and thereby reduce the mortality during transport.

Grow out: Fattening of mud crab

There are controversies to include crab fattening as a form of aquaculture (Pillai et al 2004). However, historically mud crab aquaculture probably started as crab fattening. It is a way to improve the value of catch by holding them for a short period to improve the marketability (Overton and Macintosh, 1997). Grow out culture of mud crab in many cases merely fattening of wild crabs in ponds or cages as little as 20 to 30 days. The terminology of fattening has received a confused meaning among public. Fattening is only intended to allow crabs to develop firm flesh and hardened shells. In some cases to produce egg crabs; here female crabs that show early signs of gonad development are held until the gonad get matured. Essentially fattening improves the quality of crab meat and in turn the marketability of the products.

Description of farming: General farming practices are identical to the grow-out based on juvenile crabs except in the culture duration and size characteristics of the stocking material. Recently molted crabs that are unacceptable to the export market are used as 'seed' for stocking. The pond enclosures are smaller than the juvenile rearing ponds (100-200 m^2). However pond netting and fencing are essentially identical to juvenile based grow-out system. The animals are fed with molluscan or fish by catch at the rate of 5-10% of biomass. Water is replenished once in 15 days depending on the availability of water source. Selective harvesting is carried out, and thus, fattening program is continuous through out the year. Performance of mud crab reared for one month in Chilka lagoon is given in the Table 2.

Table 3. Summary of the experimental fattening of mud crab conducted in Chilka lagoon Orissa

Parameter	*Value*
No of crabs stoked	61
No recovered	52
Mean initial weight (g)	519
Mean final weight (g)	529
Mean percent weight gain	2

Pond fattening is found to be economically viable aquaculture form through out the regions where it is being operated. After pond fattening, the market price of the crab increases to at least Rs 100-110 per kg. Taking an average price of 110 and 230 Rs per Kg for water and fattened crabs, respectively, indicates the gross profit per kg of crabs harvested is about110%.

Conclusion

Aquaculture is generally equated with the intensive salmon culture in developing countries and penaeid shrimp aquaculture in developing countries. These culture practices are generally technology driven practices, however there are aquaculture systems which can support the poverty alleviation program and can popularize through participatory approach.

The mud crab aquaculture is one of the best forms of rural aquaculture which has the potential for improving the rural villages of the tropics.

Presently crab aquaculture is predominated by raising wild caught juveniles to marketable size. Although there are several disadvantages for this form of aquaculture, for example, variability in number of animals to be utilized for grow out, no scope for further sophistication and potential effects on ecosystem stemming from mortality of bycatch and removal of prey from the food chain, mud crab farming is relevant and useful at least as a transient link between small scale aquaculture and industrialized aquaculture. The advantages of mud crab farming based on wild caught juveniles are manifold: availability of seed stock, which is naturally selected, less occurrence of disease and further broader economic benefits including the opportunities for coastal dwellers in developing countries. In addition, responsible capture and culture of wild juveniles improves the fishery of target species by circumventing the high rate of natural mortality associated wit settlement of post larvae.

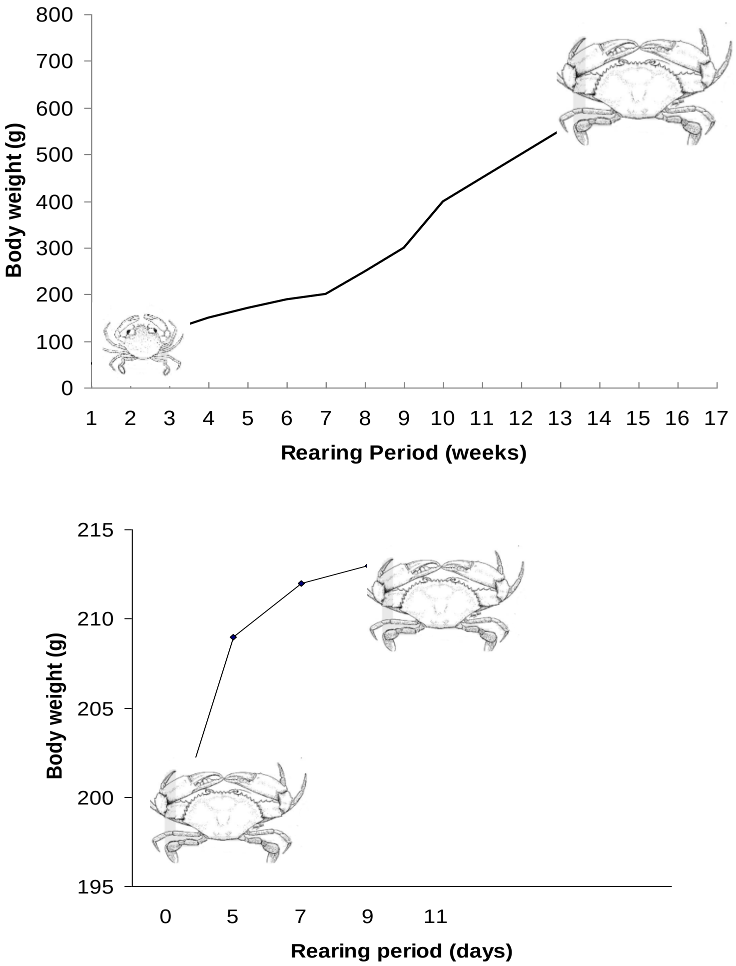

Fig 1 Diagrammatic representation of two forms of grow out culture (A) rearing from juvenile to marketable size and B) fattening of adult crab; note that size variation is not occurred in this form of rearing

Fig 2. Mud crab grow out system showing earthen mounts and hide outs (arrows)

Fig Mud crab transportation

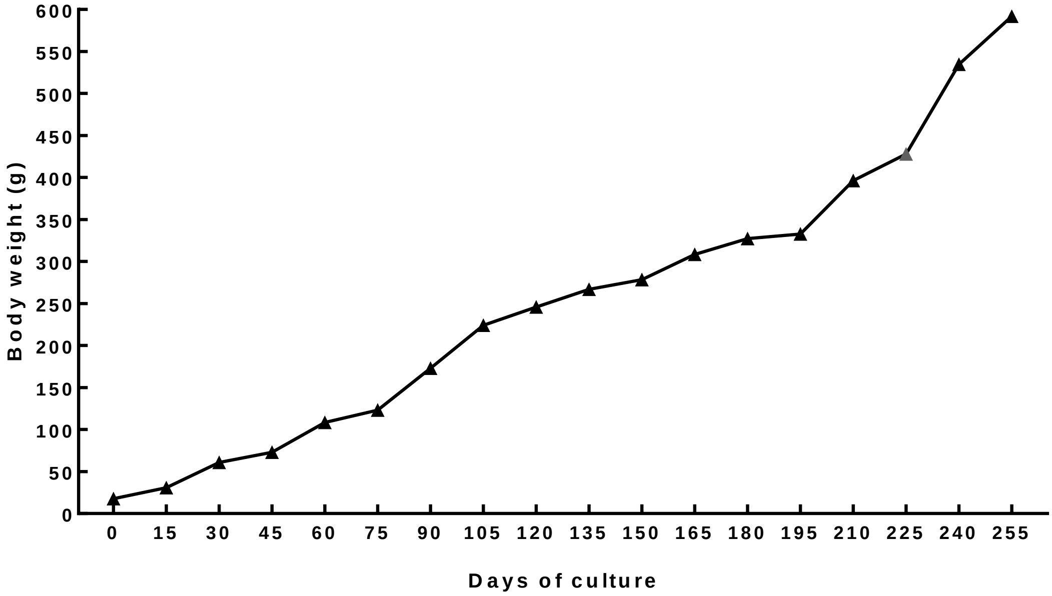

Figure: Growth of Scylla serrata reared in the farmers pond at Kakdwip (Christina 2017, CIBA Annual report

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Water and soil requirements and management in *P. vannamei* farming

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The present day shrimp aquaculture is able to thrive even under severe environmental, physical and biological stresses which are manipulated based on the understanding of experiences of successful management practices adopted by different culturists over the years. A pond with good soil and water quality will produce healthier shrimp and poor environmental conditions in pond bring in a state of stress that is unfavourable for the cultured animals but favourable for the disease causing agents. Disease is an expression of a complex interaction between host (shrimp), pathogen (bacteria/virus) and environment (pond soil and water quality). Even if the site is good with optimum soil and water characteristics, problems may still crop up by high stocking densities and use of large quantity of feed and other inputs, which lead to excessive phytoplankton production, low dissolved oxygen, high ammonia, poor bottom soil condition and other problems. Most of these problems can be avoided by proper management practices during pond preparation and culture period.

Water requirements for shrimp hatchery

One of the most important aspects with respect to both location and functionality of shrimp hatchery is the quality of water. Good quality water indicates the water capable of supporting the desired species. A thorough knowledge on the water quality requirement (Table 1) of the candidate species as well as the water quality management techniques is the essential tool for the successful hatchery operation. The most important criterion for selection of site for a penaeid hatchery is the availability of clean, clear and pristine quality seawater. The objective is to reproduce the near constant condition found in the deeper ocean where shrimp breeds and completes the larval phase of the life cycle.

Water requirements for shrimp farming

Marine shrimp are traditionally cultured in coastal or estuarine waters. The Pacific white shrimp, *Penaeus vannamei* is found in waters with a wide salinity range (1 to 40 ppt). The high tolerance of *P. vannamei* to low salinity and the year-round availability of healthy post-larvae (PL) make this species an excellent candidate for inland farming. *P.vannamei* is being cultured by farmers in sea, brackish and fresh waters. Groundwater may differ significantly in terms of its relative ionic composition compared to seawater. Most saline groundwater is deficient in potassium although other key ions such as sodium, chloride, calcium and magnesium can also vary considerably depending on the aquifer. The optimum range of water parameters is given in Table 2.

Table 1. Suggested water quality criteria for penaeid shrimp hatchery

Parameter	**Nauplii**	**Protozoea**	**Mysis**	**Post larvae**
Ammonia(NH_3-N) (µg/l or ppb)	10	17	48	100
Nitrite(NO_2-N) (mg/l)	0.11	0.29	0.45	1.36
Nitrate(NO_3–N) (mg/l)	-	-	-	<200
Dissolved Oxygen (%)	----------------------------------		>95	----------------------
H_2S (µg/l)	----------------------------------		<2	----------------------
Chlorine residue (µg/l)	----------------------------------		<10	----------------------
pH	----------------------------------		7.9-8.2	---------------------
Temperature (°C)	----------------------------------		28-32	---------------------
Salinity ppt	----------------------------------		28-34	---------------------
<u>Metals</u>				
Cadmium(µg/l)	----------------------------------		<5.0	----------------------
Chromium(µg/l)	----------------------------------		<25	----------------------
Copper(µg/l)	----------------------------------		<3	----------------------
Iron(µg/l)	----------------------------------		<300	----------------------
Mercury(µg/l)	----------------------------------		<0.1	---------------------
Manganese(µg/l)	----------------------------------		<50	----------------------
Nickel(µg/l)	----------------------------------		<50	---------------------
Lead (µg/l)	----------------------------------		<50	---------------------
Zinc(µg/l)	----------------------------------		<50	---------------------
<u>Pesticides</u>				
Aldrin/Dieldrin(µg/l)	----------------------------------		<0.003	--------------------
BHC(µg/l)	----------------------------------		<4	----------------------
DDT(µg/l)	----------------------------------		<0.001	--------------------
Endrin(µg/l)	----------------------------------		<0.004	--------------------

Table 2. Optimum water quality parameters for shrimp aquaculture

Parameters	**Optimum range**
Temperature (^oC)	28 - 32
pH	7.5 - 8.5
Salinity (ppt)	10 – 25
Transparency (cm)	30 – 40
Total suspended solids (ppm)	<100
Dissolved oxygen (ppm)	>3
Chemical oxygen demand (ppm)	<70
Biochemical oxygen demand (ppm)	<10
Total ammonia N (ppm)	< 1
Free ammonia N (ppm)	<0.1
Nitrite N (ppm)	<0.25

H_2S (ppm)	0.002
Nitrate N (ppm)	0.2 - 0.5
Phosphate (ppm)	0.1 - 0.2
Primary productivity (C/lit/day)	1.6-9.14
Plankton (No/lit)	3000-4500

Soil requirements for shrimp aquaculture

The nature of soil affects the shrimp production and hence one should have well acquaintance with the properties of soil. In India, aquaculture ponds are located under different agro-climatic conditions and brackish water aquaculture is generally being done on salt affected soils or coastal soils. Generally acidic soil and acid sulphate can cause low pH and high sulphide production respectively, unless proper management practices is followed, these soils are not suitable for aquaculture.

The soil requirements for brackishwater aquaculture are given in Table 3. Soil texture refers to the relative percentage of sand, silt and clay in the soil and has direct bearing on the productivity of the ponds. Clayey soils are best suited for building ponds as they have good water retention capacities. Sandy soils are porous and are not recommendable for bund preparation. The soil textural classes suitable for aquaculture are sandy clay, sandy clay loam and clay loam. The soil pH range from 6.5 to 7.5 is best suited for brackishwater environment as the availability of nutrients like nitrogen, phosphorus, potassium, sulfur, calcium and magnesium is highest under this range. The availability of micronutrients like iron, manganese, boron, copper, chlorine and zinc is higher under acidic pH than under neutral or alkaline. Since the requirement of micronutrients is less, it is sufficient to maintain the pH at 6.5 to 7.5. The most important index of soil fertility is soil organic matter. The presence of organic matter increases aeration, nutrient supply, reduces seepage loss, turbidity and acts as antioxidant. The microbial activity mainly depends on the organic matter content. In brackishwater aquaculture, soils with high organic matter are desirable. A productive soil should have calcium carbonate content of more than 5%.

Table 3. Soil requirements for shrimp aquaculture

Parameter	**Optimum Range**
pH	6.5-7.5
Organic carbon (%)	1.5-2.0
Available nitrogen (mg/100g)	50-70
Available phosphorus (mg/100g)	4-6
Calcium carbonate (%)	>5.0
Electricl conductivity (dS/m)	>4
Exchangeable acidity (%)	20-35
Depth to sulfidic or sulfuric layer (cm)	50-100
Clay content (%)	18-35
Textural class	Sandy clay, sandy clay loam and clay loam

Pond water and soil quality management

In view of the observed effects of environmental stress on immune system of cultured shrimp, the management strategies should include, maintaining optimum condition of pond environmental parameters. The water quality variables affecting shrimp survival and growth are determining factors for disease outbreaks. Poor water chemistry leads to deteriorate water quality, which causes stress to the organisms being raised. Regular monitoring of water and bottom soil in culture ponds for pH, DO, ammonia, nitrite and H_2S is the key in protecting the losses due to diseases.

A. Intake water treatment

Water treatment is necessary during pond preparation for the maintenance of good water quality at later stages. Water from the source should be filtered through 60µ filters to prevent the entry of parasites and crustaceans that are carriers of diseases. Reservoir has to be integral component and should be attached to grow-out ponds for sedimentation to settle organic loads and silt and chlorination treatment. Water has to be pumped in the grow out pond after 12 days of treatment, at which time, the permissible levels of chlorine residuals should be less than 0.001 ppm. Intense aeration, addition of 1 mg/lit of sodium thiosulfate for every mg/L of chlorine and exposure to sunlight are some of the practices to remove residual chlorine. Inorganic turbidity should be removed by providing sedimentation in the reservoir pond before water is taken into production ponds. Grow out pond should be filled with water from reservoir pond.

Source water filtration

B. Water exchange

Traditionally the management of water quality is through water exchange to reduce organic and to flush excess nutrients and plankton (cyanobacteria) out of the pond. Periodic partial removal of cyanobacteria and algal blooms by flushing or scooping out the scum facilitates optimum density and prevents sudden die-off of the bloom. However, due to increasing farm density, deteriorating intake water quality and rise in viral diseases, the use of water exchange as a method of pond water quality management is questionable. This practice increases the operating costs due to high water and energy consumption, and the lower retention time of nutrients within the culture systems, which would otherwise be available for biogeochemical recycling by bacteria and phytoplankton, thereby increasing the availability of natural food. Minimisation of water exchange will prevent viruses and carriers/bacterial pathogens from entering the ponds and reduce the possibility of disease transmission into shrimp ponds. This also led to the reduction of wastewater discharges and only the wastewater during harvest needs to be treated. But the reduction of water exchange requires closer control of water quality parameters such as pH and ammonia, effective sediment management, careful control of feeding and reduction of stocking density. However, improperly managed closed system increases the risk of stressful rearing conditions, bad water quality and diseases in ponds. Hence, the best water management option available to farmers is limited water exchange from treated reservoir, which enables good water quality conditions in ponds, while reducing the potential of disease introduction to the farms through intake water.

C. Aeration

In a typical black tiger shrimp pond, low rpm (revolution per minute) aerators may suffice but those with high rpm are required for *P. vannamei* culture. Paddle wheel aerators are commonly used and the newer ones such as the long arm aerators and spiral aerators can circulate oxygen to the pond bottom and apply more efficient aeration. In general, aeration to achieve more than 4 ppm of DO is related to production targets, stocking density, feed usage and salinity. Manage the concentration of DO in pond waters are very closely related to the amount and type of phytoplankton, the number and condition of the existing aerator, shrimp biomass, total organic matter content in the pond, and bacterial activity. Generally, one horsepower is suggested for 500 kg production and 50 PL/m. The placement of aerators is important to prevent localized deposition of sludge. Maintaining sufficient level of DO facilitates oxidation of ammonia to harmless nitrate by nitrifying bacteria.

D. Feed management

The practice of providing food for the shrimp is trade-off between food source and water quality in the pond. It has been estimated that as much as 0.4 ppm ammonia can be added to the system for each 100 kg of feed used. Overfeeding, even in one feed can lead to sudden increases in ammonia, sometimes called ammonia spikes, a few hours later. These spikes can often be missed during daily or weekly sampling of water for ammonia levels. Thus, it is a prudent management strategy to reduce ammonia in ponds, even at lower pH. Feeding quantity should be strictly controlled, according to the weather, water quality, containing shrimp density and the actual flexibility to adjust food intake and other factors, so that smaller meals and scientific feeding.

E. Pond bottom soil management

Pond bottom management is very important because most of the shrimp activities performed in the pond bottom. Pond bottom is a feeding area which is also where the accumulation of dirt as a result of the culture process. Keeping the pond bottom clean will indirectly protect water quality and shrimp health.

i. <u>Pond preparation</u>

Before initiating a second crop in a pond after previous crop harvest, the pond has to be prepared for stocking the shrimp post larvae.

ii. <u>Draining of ponds</u>

The first step in pond preparation is draining the pond after harvest of the previous crop. Removal of waste by draining and drying of the pond bottom after the production cycle are some of the steps to be followed for keeping pond environment clean. This could be done either by pumping or draining through sluice. For effective and complete drain, the pond should be designed in such a way that the bottom must have a gradual slope from the inlet gate to drain gate. The effective slope is 1:500. After draining, pond should be desilted.

iii. Pond mud drying and sediment removal

In this method after the final drain harvest, the pond bottom is allowed to dry and crack, primarily to oxidize the organic components left after the previous culture. The pond bottom is sun dried for at least 7-10 days or until it can support a man's weight without subsiding and the soil should crack to a depth of *25* - 50 mm. After drying, the waste can either be removed manually or with machines. Drying and cracking of pond bottom enhances aeration and favours microbial decomposition of soil organic matter. The effect of drying period on the viability of white spot syndrome virus (WSSV) in pond bottom soil after harvest indicated that 19 days drying make the WSSV unviable and hence recommended a minimum of three weeks drying between successive crops to avoid WSSV infection.

Generally after the crop harvest, water draining is not uniform throughout the pond bottom in most of the farms and it takes more time for draining compared to the other portions on the pond bottom. To enhance the oxidation of such wet patches nitrate salts at 20-40 g/ m^2 could be applied. The nitrate salts enhances the organic matter degradation by acting as nitrogen for microbes.

iv. Eradication of predators and unwanted species

After the crop is harvested, undesirable species like pests, competitors and predators remain in the ponds, which should be removed. These species include finfishes, crustaceans and molluscs. Elimination and control of undesirable species from shrimp culture pond is very important to get good yield. There are two methods to control the undesirable species. Physical method is effective in drying the ponds. Unwanted organisms are removed from the pond by drying of the pond bottom. Direct sunlight helps to disinfect the light sensitive pathogenic microorganisms (bacteria, fungus, virus) and to desiccate egg, larval and adult stages of predators. It also helps in elimination of undesirable algal mats of filamentous algae. In cases, where complete drying is not possible, organic and biodegradable, piscicides such as mahua oil cake, saponin etc. can be used.

v. Liming

Liming of the pond bottom is one of the most important items in pond preparation to keep the pond environment hygienic for sustainable shrimp production. Liming is an agricultural practice that has been adopted by fish/shrimp culturists and lime materials used in aquaculture are the same that is applied in agriculture. As a practice lime materials such as agricultural limestone ($CaCO_3$, quick lime or unslaked lime (CaO), and hydrated lime or slaked lime [$Ca(OH)_2$] are commonly used in agriculture. Besides above lime materials other materials such as dolomite, calcite, seashell and hydrated granules gained importance in shrimp culture. Most of the shrimp/fish farmers use these materials depending on local availability. Liming can be done in two ways, by broadcast over dried pond which includes the dike inner walls and by mixing with water and spraying over the pond bottom

The commercially available lime materials from market have to be analysed for their neutralisation value. The term "neutralising value" refers to the relative ability of lime materials to neutralise acidity. Pure calcium carbonate is assigned a neutralisation value (NV) of 100 per cent and is the standard against which various lime materials are compared. Thus, the neutralising power is nothing but a statement of its strength with reference to calcium carbonate or its calcium carbonate equivalent (CCE). The finer the lime material, quicker is the reaction with the soil. Different lime materials available in the market vary considerably in their particle size. Hence, a fineness guarantee is desirable. A mechanical analysis is made by the use of different mesh sieves to calculate the fineness factor or efficiency rating (ER).

vi. Management of pond bottom during culture

During culture, the feed not eaten by the shrimps and carbonaceous matter, suspended solids, faecal matter and dead plankton etc. settle at the pond bottom. To understand the condition of the pond bottom, the parameters to be monitored regularly are: pH, organic carbon content and redox potential. Reduced or anaerobic sediments may occur at the pond bottom of heavily stocked pond with heavy organic load and poor water circulation. Under anaerobic condition of the pond bottom, reduced substances such as H_2S, NH_3, CH_4 etc. are formed which are toxic to benthic organisms. Among the pond bottom quality indicators redox potential can be measured in situ by using portable redox meter or probe. The redox potential (Eh) of mud should not exceed -200 mV. The following management practices are recommended to improve the pond bottom quality.

Measurement of soil redox potential

- Central drainage canal in the pond may also help in the removal of organic waste periodically.
- Water circulation by water exchange, wind or aeration helps to move water across mud surface and prevent the development of reduced condition. Bottom should be smoothened and sloped to facilitate draining of organic waste and toxic substances.
- Bottom Raking - The oxygenated water and surface should be always in contact for the purpose of maintaining the oxidized layer. Stirring the bottom layer by manual raking and chain dragging are the common methods to improve the cont[illegible] the oxidized layer.

F. Use of chemicals, disinfectants and probiotics

Various chemical products and probiotics have been recommended for reducing the load of harmful bacteria in the pond and to improve water and soil parameters in optimum range. There is very little evidence for the efficiency of these compounds. Effective use of scientifically proven products helps in maintaining the optimum pond environment. Most of the recommended substances are broad-spectrum disinfectants including quaternary ammonium compounds (Benzalkonium chloride), buffered iodophores and calcium hypochlorite. Zeolites, although widely used, have been shown in several studies to be ineffective in reducing ammonia at salinities above 1 ppt due to competition with other ions in salt water such as sodium, potassium, magnesium and calcium. Application of gas adsorbents or probiotics to adsorb or reduce ammonia and H_2S are being practiced. However, application of probiotics can give inconsistent results due to wide differences between bacteria counts and strains, differences in the environmental conditions in which they are used, and the slow growth of many probiotic bacteria strains in ponds. Generally the deficiency of mineral is seldom observed in brackish water, whereas after introduction of *P.vannamei* in low saline, farmers are very keen on mineral application. In order to calculate the desired mineral levels at different water salinities, the water salinity (in ppt) is to be multiplied by the factors shown for each mineral (Table 4).

Table 4. Concentration of minerals at different salinities
(Calculated based on the factor for 1 ppt salinity)

Minerals	**Salinity**		
	1 ppt	5 ppt	10 ppt
Calcium (ppm)	11.6	58.0	116.0
Magnesium (ppm)	39.1	195.5	391.0
Potassium (ppm)	10.7	53.5	107.0
Sodium (ppm)	304.5	1522.5	3045.0

Wastewater management

Coastal Aquaculture Authority has made wastewater (effluent) treatment system as mandatory for *P.vannamei* farming irrespective of the size of the farm. Shrimp farm wastewater after harvesting has to be treated and disinfected by chlorine before discharge to open water sources. The wastewater from the pond may be allowed into a settlement pond before letting it into the environment so that suspended solids may settle at the bottom and the sludge has to be removed periodically. Shrimp farm wastewater is rich in nutrients such as nitrogen and phosphorus and can be utilised by integration with other aquaculture production systems. Culture of finfish, molluscs and seaweeds in the wastewater from shrimp ponds can remove nutrients and particulate organic matter. To reuse the water, reservoir is required to ensure that water treated along the treatment system is within the standards acceptable for culture.

Conclusion

Sustainability of aquaculture depends on the maintenance of a good environment. The well-designed and implemented BMPs should increase the efficiency and productivity by improving the soil and water quality, reducing the risk of shrimp health problems, reduce or mitigate the impacts of farming on the environment. Regular monitoring of environmental parameters and timely mitigation is the key to protect potential losses due stress and opportunistic bacterial infections. The understanding on ecological process occurring in shrimp culture ponds through regular monitoring will help to solve some of the disease issues in shrimp farms.

Aquatic animal health management in brackishwater aquaculture-Indian scenario

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Brackishwater farming in India during the past four decades has evolved from age-old traditional systems of 'pokkali' in Kerala, 'bheries' in West Bengal, 'gheris' in Odisha, 'khar lands' in Karnataka and 'hazans' in Goa coasts to modern semi-intensive practice. During the 90's brackishwater aquaculture development received a huge impetus on semi intensive shrimp farming with the demonstration project by The Andhra Pradesh Shrimp Seed Production Supply and Research Centre (TASPARC) funded by the Department of Biotechnology (DBT), Govt. of India. Commercial scale shrimp farming started gaining roots only after 1988–1989 and the semi-intensive farming technology demonstrated production levels reaching 4–6 tons/ha. Giant tiger shrimp (*Penaeus monodon*) became the mainstay of brackishwater aquaculture in India and the area under shrimp farming showed remarkable growth rate till 1995.

During the initial years of semi-intensive brackishwater aquaculture development in the country, disease outbreaks linked to crop failures were not there, while disease conditions such as soft shell syndrome, bacterial septicaemia caused limited problems in grow-out aquaculture. Major setback due to disease struck during 1994 due to outbreaks of white spot syndrome or white spot disease (WSD), caused by one of the largest double stranded DNA viruses, the white spot syndrome virus (WSSV) and has been devastating our shrimp farming sector since then. The profitability and sustainability of tiger shrimp farming was in question due to the new disease threat and hike in input costs and decreasing sale value. The development of Indian shrimp farming was getting affected and farmers were looking for an alternative model. Many brackishwater shrimp farmers shifted to scampi farming in brackishwater ponds, which also suffered setbacks due to white tail disease (WTD), caused by an RNA virus, the *Macrobrachium rosenburgii* nodavirus (*Mr*Nv), and the scampi farming never recovered. Later on since 1998, the black tiger shrimp farming continued to face challenges such as loose shell syndrome (LSS), followed by the monodon slow growth syndrome (MSGS) since 2004 in black tiger shrimp farming.

Diseases in shrimp farming

P vannamei is known to be vulnerable to a wide range of viral diseases, and the reports of mass mortalities and failure of the culture system have also been recorded. The common diseases of economic importance reported to affect vannamei include, WSD, infectious hypodermal and hematopoietic necrosis (IHHN), Taura syndrome (TS), yellow head disease (YHD) and infectious myonecrosis (IMN). WSD caused US $6 billion loss since the year 1992-1993 to Asian shrimp aquaculture, while in America, WSD has caused US $1–2 billion loss. IHHNV has caused about US $ 0.5–1 billion loss in America. The impact of TS on the shrimp-farming industry in the Americas was estimated to be US $ 1–2 billion up to 2001 while in Asia it is of $0.5–1 billion (Lightner, 2003). An unpublished data from Brazilian shrimp farmers' association has estimated that the loss due to IMN from 2002 to 2006 in Brazil exceeded $100 million. In Indonesia this IMNV caused significant losses exceeding $1 billion by 2010. A total of $100–200 million loss has occurred mainly because of this disease in America, but in Asia this disease has emerged in the year 2006, the estimated loss is about $1 billion. Significant loss from YHD amounts to $1 billion per year in Asia. A new unique disease, acute hepatopancreatic necrosis disease or AHPND earlier known as early mortality syndrome (EMS) has been devastating *P. vannamei* farms in China since 2009, Vietnam since 2010, Malaysia since 2011, and Thailand since 2012, where in 100% mortalities have been reported during the first 20-30 days after stocking. AHPND has caused ~60% drop in shrimp production in the affected region compared with 2012 and the global estimate of the losses per year is about US$ 1 billion. All these disease related issues resulted in the prediction by FAO that global supply of shrimp would contract by 15% in 2015, ultimately showing the challenges posed by disease issues alone to the dynamic industry of shrimp farming across the world.

CIBA's investigations have revealed that during the year 2016-17, the prevalence of WSD was 8.9%, hepatopancreatic microsporidosis or *Enterocytozoon hepatopenaei* (EHP) 23.6%, monodon baculovirus disease (MBV) 2.3% and IHHN 1.3% in vannamei farms in the major shrimp farming states of Andhra Pradesh and Tamil Nadu. Indian brackishwater aquaculture was free from other OIE listed diseases such as TS, YHD, AHPND and Necrotising Hepatopancreatitis (NHP). Other disease syndromes due to poor farm management such as stunted growth, white faeces syndrome (WFS), white muscle syndrome (WMS), running mortality syndrome (RMS), popularly known as running mortality syndrome (RMS) and black gill syndrome was observed in 15.17%, 16.5%, 3.4%, 2.7%, 7.5% of the farms respectively. High stocking densities, lack of crop holidays and poor pre-stocking management are attributed to several of these conditions, and these conditions can be termed as farming system related ones and not due to pathogen involvement. The information generated indicates that WSD still remains the most devastating shrimp disease in India which causes chronic regression in production. However the emergence of EHP and its impact due to its accumulation in culture environment since its detection in 2015 in India is alarming. Emergence of such new diseases in Indian aquaculture despite stringent screening of brooders at AQF facility of RGCA as post-border measure point to the clandestine use of pond reared brood stock for seed production in the country by a section of operators. Sensitive diagnostics are available to detect any form of these pathogens, however routine diagnosis yet to be adopted by hatcheries and shrimp farmers. It appears that farmers have a preconceived idea that there is no need of screening the SPF vannamei seeds. In this backdrop, CIBA initiated the programme of harmonisation of PCR diagnostics across the country as per the guidelines and intense efforts are being taken for capacity building of human resources associated with these laboratories, partnering with MPEDA and CAA.

Diseases in finfish grow-out culture in India

Presently, the Indian brackishwater aquaculture sector is dominated by shrimp culture due to its high export value. India has a great potential for brackishwater finfish culture and with the support of the aquaculture promoting agencies and R&D institutions, hatchery and farm rearing technologies of new finfish species are already being demonstrated by the farming sector for economic viability. Expansion of brackish / marine finfish aquaculture has been taking place along the coastal states of India especially in the states of Andhra Pradesh, Tamil Nadu, West Bengal, Orissa and Gujarat. As with the case of any live animal rearing issues, disease has been an issue in finfish culture sector also. Although a number of viral and bacterial pathogens have been reported from other finfish farming nations, only viral nervous necrosis (VNN) has been reported to be present in India, mainly causing mortalities in the larval stages of seabass. Among the bacterial pathogens, *Vibrio* spp are reported to be associated with diseases in brackishwater finfish by some investigators.

Fish Health Management Strategies

An understanding about the environment, biota and biology of the target species along with the in depth knowledge of the disease, pathogen, disease development, diagnostics, epidemiology and control measures are essential factors in management of a disease problem. Hence, Fish health management requires a holistic approach, addressing all aspects that contribute to the development of disease. Disease outbreak is an end result of negative interaction between pathogen, host and the environment. Hence, management of disease problems must be aimed towards broader ecosystem management with a view to control farm-level environmental deterioration and to take preventative measures against the introduction of pathogens into the aquaculture system. The emphasis should be on better management for prevention, which is likely to be more cost effective than treatment, involving both on-farm management and the management of the environment. Steps must include reducing the use of chemicals and drugs. Regulations with respect to land and water usage, environmental protective measures, inputs that go into the aquaculture systems, farm-wise and region-wise must be put in place by the Government for disease management of aquatic animals and sustainable development of aquaculture at large. In addition, research and development, training programs, extension, and information exchange would help achieve the objective of disease prevention and control in aquaculture effective. The FAO's Code of Conduct for Responsible Fisheries would provide a good base for the national and international cooperation in harmonizing aquatic animal health management activities.

New generation approaches such as Surveillance techniques, Contingency planning and Import Risk Analysis (IRA) are gaining importance as critical tools in the health management strategies of aquatic animals for quick and effective response to new disease outbreaks. Functioning of a national level (each country) body with necessary responsibility and mandate to implement a 'national health management strategy' or 'health management regulation' on the basis of existing international standards, guidelines or recommendation from FAO, OlE and NACA and WTO must be there in issues related to aquaculture and aquatic animal health management for the region.

Aquatic animal health monitoring and aquatic animal disease surveillance

The term aquatic animal health monitoring is nothing but comprehensive collection, analysis and dissemination of information about diseases that are known to occur in the population which is being monitored. It is used to evaluate the frequency and trends of diseases, the risk factors associated with it and its economic impacts. Monitoring programs are used in conjunction with disease control and eradication programs frequently. The term *surveillance* implies an active process in which data are collected, analysed, evaluated and reported to those involved with a goal of providing better control of a disease or condition. It aids in detecting an exotic or new disease within a given population. Disease surveillance programs are designed to argue freedom from specific diseases and should always elicit action in the event of an exotic disease introduction. Important questions often asked as part of the surveillance programs include: (i) is the frequency of the disease remaining constant, increasing or decreasing?; (ii) are there differences in the geographical pattern of the condition?; (iii) does the disease have any impact on productivity and / or profitability?; (iv) is the disease absent from a particular species / strain, region, or nation?; and (v) is a control or eradication program cost-effective?

Due to the wide variety of species cultured, the pathogens and management systems, is necessary to establish surveillance systems; these should be designed to demonstrate freedom of aquatic animals from infectious diseases, taking into account the definition of the population, including any sub-populations that should be targeted to improve the probability of detecting disease, clustering of disease, documentation of the methodology used, survey design and data analysis procedures, the test or test system being used, the design prevalence or minimum expected prevalence in the presence of disease, sampling approaches, and quality assurance systems. Surveillance has been recognized as one of the key elements of any animal health policy, giving priority to preventive approach, early detection and rapid response.

The National Surveillance programme has been initiated through a network of fisheries research institutions active in aquatic animal health since 2014. The project was conceived with the following objectives: (i) Investigate and detect new and exotic infectious disease outbreak in aquatic animals; (ii) Provide evidence of freedom from diseases of concern within a defined geographical area; (iii) Collect information on the distribution and occurrence of diseases of concern; (iv) Assess progress in control or eradication of selected disease pathogens. About 24 leading national institutions covering fourteen states with passive and active surveillance in more than 100 districts are carrying out disease surveillance in farming of shrimp, carp, catfish, tilapia, ornamentals, cold water species, freshwater prawn and molluscs. A Technical Advisory Committee (TAC) has been constituted to oversee the implementation of the project with NACA, Bangkok as a special invitee.

Aquaculture biosecurity

Biosecurity is a broad concept and the application of biosecurity concepts to shrimp aquaculture will contribute significantly to reduce losses due to diseases and make this sector more sustainable and environmentally responsible. Implementation of biosecurity practices is an increasingly pressing issue for fisheries and aquaculture managers, considering the importance of this sector in terms of food security and economic development of the people. Biosecurity measures implemented appropriately can be a cost- effective way of managing disease risks. Adopting quarantine measures for broodstock prior to their use, adopting best management practices (BMPs) and standard operating protocols (SOPs) by implementing good sanitary practices, treating water before use, optimizing stocking density of larvae and maintaining good water quality will help in achieving biosecurity in hatcheries. At farm level, implementing biosecurity plan requires modifying existing farms and management routines. Main preventive measures at pond / farm level include proper pond preparation to eliminate pathogens and their carriers, treatment of water in reservoirs to inactivate free viruses and kill virus carriers, water filtration using fine filters to keep carriers out, closed zero-water exchange systems to avoid contamination from source water. Shrimp ponds with a history of disease outbreaks have a greater likelihood of future disease outbreaks, and hence, special attention is required during pond preparation.

Aquatic animal quarantine

Aquatic animals are widely translocated across countries for enhancing aquaculture productions and species diversification. Such trans-boundary movement of live aquatic animals has the risk of introduction of new diseases. Responsible fisheries emphasizes the need to minimize the risk of disease transfer and adverse effects on wild and cultured stocks associated with the introduction of non-native species and transport of eggs, larvae, broodstock and other live organisms. Presently two amphibian, ten fish, seven molluscan and eight crustacean diseases have been listed by the World Organization for Animal Health (OIE). In mollusks, parasitic diseases are important, while in fish and crustaceans viral diseases are cause of concern. Whether a listed disease is due to a virus, fungus, bacterium or a parasite, the occurrence of the disease may adversely affect international trade among trading partners that have, or do not have, the listed disease. India established its first state of the art AQF in the year 2009 at Chennai to facilitate *P. vannamei* broodstock import in India. However, India needs to establish separate AQFs for quarantine needs of various aquatic species such as ornamental fish, candidate finfishes such as seabass, corals, being proposed by the private sector for import. These facilities can be created under PPP mode, where central agencies, state Govt., research institutions and the private players can play a harmonised role. The import risk analysis (IRA) of CIBA clearly revealed that the aquatic quarantine at the importers' facilities was highly risky and recommended low-risk options to the Govt. of India to i) establish quarantine facilities under public sector with restriction on the culture practices and ii) establishment of SPF broodstock multiplication centre cum quarantine under public-private partnership with restricted permits for culture, as is presently being done with Pacific white shrimp. At large, the Quarantine and Biosecurity must be vested with the Govt agencies and Institutions as done in all other developed nations for effectiveness and delivery as per the regulations of the country.

Aquaculture Certification

Fish is one of the most highly traded food commodities globally. According to FAO, global trade of fish and fishery products reached $136 billion in 2013. EU, US and Japan together account for about 70% of international fish imports and most fish exporting countries are trying to access these markets. Import requirements in these countries are very stringent in terms of quality, safety and fair trade practices. The World Trade Organisation (WTO) Sanitary and Phytosanitory (SPS) and Technical Barriers to Trade (TBT) agreements provide a framework for international trade including that of fish and fishery products. These are based on principles of (a) sovereignty, (b) harmonization, and (c) equivalence. According to these agreements, WTO member countries have the right to take measures to protect animal health and consumer health, based on a scientific risk assessment performed according to internationally accepted practices. The member countries are expected to harmonise their standards by those adopted by international organisations. For food safety, Codex Alimentarius Commission standards and for animal heath, OIE standards have been recognized in the SPS agreement. Issues related to food safety such as food hygiene, maximum permissible limits for contaminants, residues of veterinary drugs are generally part of national regulations. Examples include EU regulations and USFDA regulations. In India, the Food Safety and Standards Authority of India (FSSAI) is responsible for developing and implementing national food safety related standards. Fish processing establishments, that already implement the mandatory HACCP based quality and safety management programme, are obliged to get certified by private certifying bodies like the British Retail Consortium (BRC), International Featured Standards (IFS), Food Safety System Certification (FSSC) 22000, Safe Quality Food (SQF) Institute certification and others. The EU requires that the national regulatory requirements are harmonized and equivalent to EU requirements. The US and Japan follow different procedures and it is the responsibility of the importing company to ensure that the operators in the producing country meet the USFDA / Japanese Ministry of Health requirements. India has been successful in accessing all these major markets in addition to other regional markets as is evident by growing exports of Indian seafood. Keeping this growth trend requires sustained efforts to maintain quality standards as required by the importing countries. The Export Inspection Council of India although specifies the requirements of traceability for fish and fishery products, it is essential that traceability to be established at all stages of production, process & distribution to get the confidence of the food business operators. However, considering costs involved in aquaculture certification which has to be borne by the producer (farmer), it is essential that India develops its own aquaculture certification programme for the benefit of the sector, keeping in view more than 80% small aqua farmers, where institutions such as CIBA, MPEDA and CAA could play the lead role.

Concluding remarks

While Indian aquaculture sector is booming after the introduction of exotic specific pathogen free Pacific white shrimp, trans-boundary diseases could limit this growth and affect sustainability. Maintaining strict biosecurity in aquaculture is a challenge, but is not an impossible task. Aqua farmers have to be educated on biosecurity principles and adoption of best management practices on a regular basis to maintain sustainable productivity. While India has shown its capacity in establishment and successful operation of AQF for *P. vannamei* broodstock since the year 2009, there is a need to establish similar separate AQFs for quarantine of various aquatic species proposed for import by the private sector time to time to safeguard India’s aquatic biosecurity. This will be driven by various factors like production profitability, investment and development issues, and new threats of emerging health problems, resource protection, food security, trade, consumer preference for high quality and safe products. For sustaining international trade, aquaculture certification needs to be evolved within India based on the FAO Technical Guidelines for Aquaculture Certification as the benchmark.

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Formulated feed for shrimp farming

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What is meant by Feed quality?

The quality of the feed is the single most factor which has got major influence on the successful operation of the aquaculture enterprise as feed alone contributes about 60% of the operating cost in modern days aquaculture using improved traditional and semi intensive farming practices. A good definition of quality is difficult to explain and in the common parlance quality is about meeting the needs and expectations of customers. If we are able to assess the quality of the feed before it is fed to aquatic animals it will save us lots of trouble and money. Hence, attempt has to be made to assess the feed quality as quickly as possible. There are different type's qualities which can be done easily and quickly.

1. Physical quality:

This is the important quality normally used by farmer for evaluating the feed. The nutritionist/ farmer or the mill manager should train himself to use all his five senses to identify the changes in the nature of finished feed. The appearance of the feed will reveal its quality and the colour will depend on the type of the ingredient used. However in certain conditions change in the colour of the feed is an indication of the storage condition, presence of toxins etc. The size of the pellet should be uniform and it should be free from other contaminants with powder percentage to the acceptable level. Smell is the important indicator in shrimp and carnivorous fish feed. A good fishy smell indicates that it contains considerable level of marine protein sources and hence the feed will be highly attractable and palatable for the shrimp and fish. Water stability of dry shrimp/fish feed pellets is the crucial physical quality which is determined by the loss in weight of pellets kept in water for a specified time interval. The loss in weight of pellets indicates the stability, higher the loss poorer the stability. Normally the water stability for shrimp feed is not less than 2 hours and 1 hour for fish feed.

2. Chemical quality

Even though physical quality of the feed will indicate the worthiness of the feed the actual nutritional quality can be assessed only by subjecting it for chemical/laboratory evaluation. The feed can be analyzed for proximate and chemical composition and ensured that the nutrient contents are in the desirable level for the candidate species.

3. Biological quality

The physical and chemical quality n will not be able to reveal everything about the ingredient sometimes when all the tests prove ideal nature of an ingredient but it may cause problems to the fish and shrimp, hence this particular feed may be subjected for biological evaluation to know its suitability to the candidate species.

4. What is feed management?

Feed management means control and use of feed for aquaculture operation in such a manner that the utilization of feed is optimum with minimum wastage, negligible impact on environment, achieving best feed conversion ratio (FCR) and maximum growth of fish and shrimp and production. Such feed management practice if adopted, aquaculture production will be not only economical and profitable but also sustainable and eco-friendly. A best can produce poor results if the feed management is poor. On the other hand a moderate feed can produce best results under good feed management.

Most of the feed suppliers provide feeding charts for feeding fish and shrimp during the period of culture operation. These tables may be prepared based either some experiences or based on theoretical models. Since most of the feeding charts are based on size of fish and biomass in the culture pond still errors occur because accurate estimation of biomass in a pond is very often not possible correctly. In many farms excess feeding may occur due to this error. In some cases farmers may be over enthusiastic in achieving faster growth may over feed the stock leading to poor feed management.

5. Rate of feeding

Even though there are some investigations on the quantities requirements of feed in relation size and stage of the growing fish/shrimp still research on these aspects is needed for making the feeding tables more accurate. 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. Suppose if W grams is the average weight of the stoked animal and if there are A number of animals in the pond then the total biomass in the pond is W x A grams which is equal to W x A/1000 kg. If feed is to be given at 10% of body weight then the quantity feed required per day is (W x A/100) x10/100 kg.

In pond to estimate the biomass accurately is not possible. Generally periodically (once a week or 10 days) using a suitable net, sampling of the fish/shrimp and the average weight of the animal is calculated. Total biomass is calculated by multiplying the average weight by the number of animals surviving at that time. This is mainly by done by counting the number of animals caught per each netting and estimating the total number of animals taking into accounts the area covered by each netting and the total area of the pond. Some times the number of animals surviving in the pond is approximately estimated by giving a margin of 5 –10% mortality per month on the total number of animals initially stocked.

The alternative method of feeding is not by calculating the daily ration but by leaving the fish on self-demand feeding conditions. When the fish is hungry it will approach the demand feeder for its food requirements. It was observed that fish quickly learn how to obtain feed. The growth of fish also is good with best FCR and minimum wastage of feed. This method works best with finfish farming. Mechanical demand feeders and feed bags suspended at different places in pond are used in this method feeding.

Floating pellet feeds for finfish have the advantage in controlled feeding. Since the feed floats on the surface of water, the active feeding by fish can be directly observed and the consumption of feed can be monitored. Based on the observations the quantity of feed to be broadcast can be regulated.

6. Schedule and frequency of feeding

The total quantity of feed required in a day should not be fed at time. Scheduling and frequency of feeding greatly help in successful feed management. Time schedules for feeding the fish may be fixed such 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. Some species are more active during night and should receive comparatively larger portion of the ration. Observations and experiences show that frequent feeding of small portions of the ration seems to help in better utilization of the feed and there by lead to efficient FCR. The daily ration can be offered at every 2- 4-hour interval in divided doses. There must also a mechanism in each case to monitor the feed consumption and offering of the next scheduled dose should be regulated according to the consumption from the previous feed offered. Regular observations and experience help in mastering the management of feeding in a culture farm.

7. Feeding shrimp in grow-out ponds

The quantity of feed required in a day for feeding shrimp is estimated based on biomass in the culture pond. To start with feed is offered at 15 – 20% of body weight. As the shrimps grow, it is gradually reduced and brought down to 2-3% towards the end of the culture period. A model chart for feeding is given in Table 1. The entire quantity of feed required for a day in a pond should not be put at one time. The shrimps should be offered feed at every 3 - 4 hours in small doses. This helps in better utilization of feed and reduces wastage. Shrimps are active feeders during night, hence large doses may be offered in the evening and during night. Keeping the feed in bamboo or velon screen trays kept inside the pond at different locations is a good practice (Fig. 1). These are known as check trays. Periodically these check trays can be lifted up to check the feed consumption. A part of the feed may also be broadcasted for proper distribution. Instructions of the feed supplier with regard to feeding may be followed. Excess feeding leads to uneaten feed at the pond bottom. This will cause pollution of pond water and stimulates algal blooms, which may cause stress to shrimp. Under these conditions mass mortality of shrimp may occur. Feeding a little less does not do any harm, but feeding a little excess may be harmful and can cause heavy loss. Feed management needs experience and skill to obtain best results. Water quality in culture pond is also linked to feed management. If the water quality (such as dissolved oxygen, ammonia, nitrite, nitrate, hydrogen sulphide) in the pond is poor, even the best feed may give poor performance.

Shrimp feeds should be stored properly. Absorption of moisture during storage leads to mold growth and lowers the quality. Certain kinds of fungi (*Aspergillus* sp) produce aflatoxin, which is very toxic to shrimps. Feedstocks required for use of one month may be purchased at a time and stored in a cool and well-ventilated place. For longer shelf-life, the feed may be stored at lower temperature of 10^0 C.

Farmers should look for feeds that are as fresh as possible. Fresh feeds generally give good fishy smell. Stale smell indicates that the feed is not fresh. Water stability of feed also affects the performance of the feed. It will not disintegrate fast but also causes water pollution leading to economic loss. The feed should be stable under water at least for 2 hours. Feed should not be too hard also as it not properly assimilated the animal. Feed with poor water stability leads to poor FCR and higher cost of production

Table 1: Rate of feeding of shrimp and quantity of feed to be given in culture pond

Week after Stocking	**Weight of shrimp (g)**	**Survival expected %**	**Rate of feeding % of body weight**		**Quantity of feed to be given per day (kg)**	
			$5/m^2$ *	**$10/m^2$ ***	**$5/m^2$ ***	**$10/m^2$ ***
1	0.5	90	nil	--	nil	2.0
2	1.0	89	nil	--	nil	4.0
3	2.0	88	4.0	6.0	3.5	10.5
4	2.9	87	3.8	5.5	4.8	13.9
5	3.9	85	3.6	5.0	5.9	16.6
6	5.0	84	3.4	4.8	7.1	20.2
7	6.2	84	3.2	4.6	8.3	23.9
8	7.5	83	3.0	4.4	9.3	27.4
9	9.0	82	3.0	4.0	11.0	29.5
10	11.0	80	3.0	3.8	13.2	33.4
11	14.0	78	2.8	3.4	15.2	37.1
12	16.0	76	2.5	3.2	15.2	38.9

13	18.5	75	2.4	2.8	16.2	38.9
14	20.0	74	2.3	2.7	17.0	40.0
15	22.5	73	2.2	2.5	18.0	41.0
16	25.0	72	2.0	2.3	18.0	41.4
17	28.0	71	2.0	2.1	19.8	41.7
18	31.0	70	2.0	2.0	21.7	43.4
19	33.0	70	1.9	2.0	22.0	46.2

Feed dispensing technology for shrimp aquaculture

The cost of feed is the major operating cost in shrimp aquaculture, and may account for 50% or more. The major component of scientific shrimp farming is to provide artificial feeds at right quantity at right time according to the requirement and feeding is one of the most critical aspects of shrimp husbandry. There are many things that a farmer must do to guarantee a successful shrimp culture. Development of a feed of a high quality diet that is formulated to meet the nutritional requirements of the shrimp which is manufactured from high quality, digestible ingredients, with appropriate size and palatability for the shrimp is the most priority one. Along with that the method of feeding is also important since it influences the overall feed quantity consumption, soil and water quality of the pond and ultimately the success of the culture itself. It may not be an exaggeration to state that feeding method and management is as important as the development of the feed itself.

In India, shrimp farming aquaculture is synonymous to monoculture of tiger shrimp *P. monodon* till 2009 and the feeding of shrimp farms is being done manually The feeding method mostly in practice were hand feeding by broadcasting. Feeding tray method has been successfully practiced in Latin American countries like Brazil but not so prominent in India and Asian countries. In India the check trays are used only to see whether the feed has been consumed by the shrimps or not and not as a feeding method. .

Automatic feed dispensing and *P. vannamei* culture

The introduction of *P. vannamei* heralded the necessity of automated feed dispensing system. The automatic feeders may be more suitable in *L. vannamei* due to its feeding behaviour which is a column dweller compared to tiger shrimp, *P. monodon* which is a bottom feeder. So far in *P. monodon* culture, the use of auto feeders in shrimp farming is not common with the feeding behaviour of the shrimp the primary constraint. The stocking density allowed as per Coastal Aquaculture Authority guidelines for *P. vannamei* is up to 60

/sq.m and the potential productivity level is around 10-12 t /ha accordingly the feeding management requires greater attention.

To illustrate little further:-

For 1 ha pond with following assumption

Initial stocking density: 50 pc/ sq. m

Approx. survival: 80 %

Average body weight (ABW): 20g

Standing biomass: 8 tonnes

Feed requirement/ day is approx. = 100-150 kg/day

To distribute this amount of feed in number of pond becomes too labour intensive considering the timely and frequency (4-5 times/day) of application. It would be difficult to carry this much load of the feed and distribute it uniformly over the pond. In such situations automatic feeder will be very handy and effective. Automatic feeders and feeding systems could play a major role in the feeding of the *P. vannamei* culture and in near future it would become a necessity if intensive farming systems with more stocking density come into acceptance. It is a fact that the labour problem could be addressed significantly as handling the feed during the course of culture increases enormously especially during the end of culture in case of *P. vannamei*. These devices are indeed will help to overcome labour problems in the industry and introduce a semi-automatic process in the shrimp aquaculture industry. Moreover the biosecurity requirement for shrimp farming could be best met with the utilisation of automatic feeder. The feed quality could be also be well maintained with these systems.

Farmer's perspective of automatic feeding system

Field trips to the farmers' fields where automatic feeders were installed at Nellore in Andhra Pradesh and Cuddalore in Tamil Nadu were undertaken to assess the farmer's perspective of the automatic feed dispensing system and its applicability and utilisation. The automatic feeders installed in farmer's pond were mostly made up of FRP or powder coated with a capacity of 150- 200kg. The distribution mechanism adopted was 0.25 HP to 5 HP motor powered pipes of different length for uniform distribution. The approximate cost of the unit is around Rs. 30,000/- to 35,000/- and has been indigenously fabricated by the farmers with the assistance from the mechanic. The design of the feeder at Cuddalore farm is also more or less similar. The timer control unit is kept at the bund. In Nellore the control unit is little sophisticated with digital display.

The feeder is timer controlled and continuous feeding is being practiced from 6.00 A.M to 6 P.M with intensive feeding up to 2pm. Only one feeder is fixed in the pond irrespective of the size and shape. Since *L. vannamei* is a column dweller and it takes the feed from the place where it is applied. The thumb rule followed is one feeder per 0.4 - 0.6 ha approximately for a biomass of 6-10 tonnes. The aerators were also fixed and were in operation.

The farmers were very satisfied and they were of opinion that the FCR has been improved. Days of culture has been reduced. The sludge accumulation is very less rather negligible in pond installed with auto feeder in comparison with the manual feeding .The discharge water from auto feeder pond is of better quality. Soil and water quality has improved and pond bottom is clear. The Cuddalore farmers were also of the same view and even they feel this will lead in profitable and economical shrimp farming. Moreover if the automatic feeder is used the water stability of the feed could be of lesser duration when compared to manual feeding. The powder ratio and fissure percentage, the hardness of the feed all could be lesser for automatic feeding than for manual feeding. Above all the major advantage as stated by the farmers is the labour saving and it is very convenient and easy to operate.

The efficiency of the auto feeder could be evaluated by continuously monitoring one crop in farmer's field to check the actual FCR, soil and water quality, sludge accumulation etc. The size and shape of the pond vis –a vis the placement of feeder and the shrimp feeding behaviour also be investigated.

Design criteria for an efficient automatic feeding device

A simple design of feeding machine or an automatic feeder consist of four major components, a feed hopper, a mechanisms for feed distribution an electrical power supply for the distribution mechanism and a control unit for starting and stopping the distribution mechanisms.

Each component has to be designed with utmost care. The cost will be the prohibiting factor for wider adoption and hence energy efficient low cost feeder with efficient nutrient delivery is the need of the hour. The material for the feeder assembly needs to be corrosion resistant and durable in salinity condition. The material should be light but strong enough to face the windy conditions in coastal area. Also the feed should be supplied to the pond with ease and accordingly the hopper bottom needs to be designed. The angle of repose of the hopper should be greater than the angle of repose of the materials for easy dispensing from the hopper. Usually the angle of repose should be 50 for easy dispensing. The valve which controls the flow of feed as per the timer needs to be soundproof. The feed dispensed through the feeder should be as such that feed quality is not affected viz., feed is not broken. The installation of feeder needs much attention. It is better if it is installed at 15 m from the pond dyke so that uniform dispersal is effected and the radius of feed dispersion does not overlap with the dyke area. In case of intensive farming with *P. vannamei*, the size of the drum approximately is more than 100 kg and it should be fixed at the centre or if necessary two or more feeder of smaller size needs to be made. The loading of the feeder once in two or three days needs to be monitored properly. PLC controlled will be effective as it could regulate the amount and time of feeding based on the biomass automatically.

Though the automatic feeder has been embraced with the open arm by shrimp farmers the power availability is a major constraint and invertor is being used by the farmers. The inverter is capable of supplying power to 5 feeder unit for 5 hours and one unit cost approximately 30,000/-. If solar powered feeders are developed it would definitely useful to the farming community. Also the feeder which has been developed so far is only timer controlled one. If adjustments to the feeding regime with appropriate quantity based on life stage and it could be more useful

Conclusion

The innovation and adoption of energy efficient, easily operated automated feed dispensing unit is definitely indispensable for precision feeding and efficient nutrient use efficiency. Automatic feeding will benefit the shrimp industry in a major way.

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Shrimp farming technology advisory service through a mobile application

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Introduction

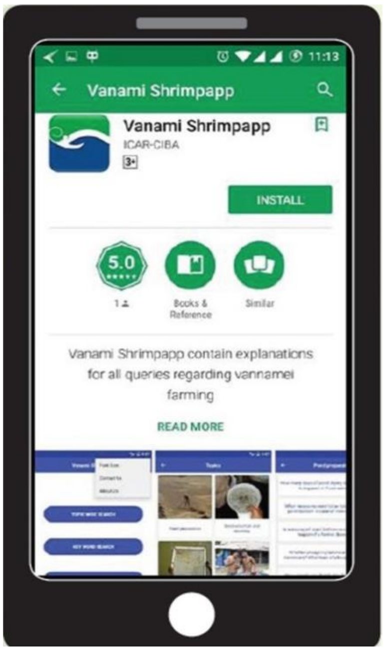
Shrimp aquaculture, rearing of shrimps in brackishwater under controlled conditions is being practised in the maritime states along the coastal line of the country. Shrimp farming is practised on commercial mode as 90% of the production is exported, earning about Rs.30,000 crores as foreign exchange (MPEDA, 2018). Being a delicate organism shrimp requires quality rearing medium, optimum feeding and health care. Shrimp farming is subject to several production risk factors from pond preparation to harvest and the farmers should have adequate knowledge and skill in Better Management Practices (BMPs) to prevent and manage them. Therefore technical information is a critical resource in operation and management of shrimp farming. In general, estimates indicated that 60 percent of farmers do not access any source of information for the advanced agricultural technologies resulting in huge adoption gap (NSSO, 2005). In India, there are about 120 million farm holdings and the number is growing year by year. To provide at least one village extension personnel to 800-1000 farm families, the requirement of field level extension personnel is estimated to be about 1.3 to 1.5 million, against the present availability of 0.1million (100000) personnel (Planning commission, GoI, 2007). According to estimates, on an average a public extension personnel spends 40 minutes per year for a farmer (Dileepkumar, 2012). There is a gap between the extension agents and farmers due to lack of manpower, poor access and ineffective service mechanisms (Mruthunjaya and Adhiguru, 2005). To minimize this gap many computer and internet based projects for providing rapid extension services were initiated but such initiatives have not been very successful, as farmers were either illiterate or not technically attuned to access information through the internet (Ganesan *et al.*2013).

In this scenario, the increasing penetration of mobile phone networks and mobile phone usage in India presents an opportunity to use it as a tool for extension service. Review on mobile phone based extension indicated that introduction of mobile phones for information sharing decreased the market price dispersion and wastage (Jensen, 2007; Abraham, 2007), significant improvements in knowledge of farmers (Kumar and Padmaiah, 2012), provided greater convenience on time saving (Surabhi Mittal and Gaurav Tripathi, 2009), reduced grain price dispersion across markets by a minimum of 6.4 percent and reduced intra-annual price variation by 10 percent (Aker, 2008), enhanced the access to technology information and reduced communication costs for the farmers (Mahedi Hasan, 2015). Similarly extension services delivery through mobile phones has significantly improved the amount, quality and speed of the services which in turn led to higher production level and income status, and reduced the gap between the better off and disadvantaged farmers (Bolarinwa and Oyeyinka, 2011; Xiaolan Fu and Shaheen Akter, 2012; Jhunjunwala *et al.* 2013). Mobile based agro-advisories a powerful means of increasing access to quality information to farmers would play a significant role in reducing the information gap and information asymmetry between the farmers (Reddy *et al*.,2017).

Mobile phones and networks available with shrimp farmers

The growth of mobile phones in India has been phenomenal during the last one decade. Internet and Mobile Association of India (IAMAI) reported that India had 934.6 million mobile phone subscribers in 2017. The number of mobile internet users in India is projected to double and cross the 300 million mark by 2017 and the number of smartphone users is expected to reach 369 million by 2018. Our study has shown that 66% of the shrimp farmers were smart phone users which mean that they were mobile internet users. Most of them (63.83%) used Android platform which should be kept in mind while developing any mobile phone based application for shrimp farmers. Most of them (87%) had networks other than BSNL and 91% of farmers preferred English as the language of interaction. Shrimp farmers were relatively better educated and afford to have advanced mobile phones, therefore, mobile phone based information exchange could be a viable strategy to provide information and services and interact with shrimp farmers.

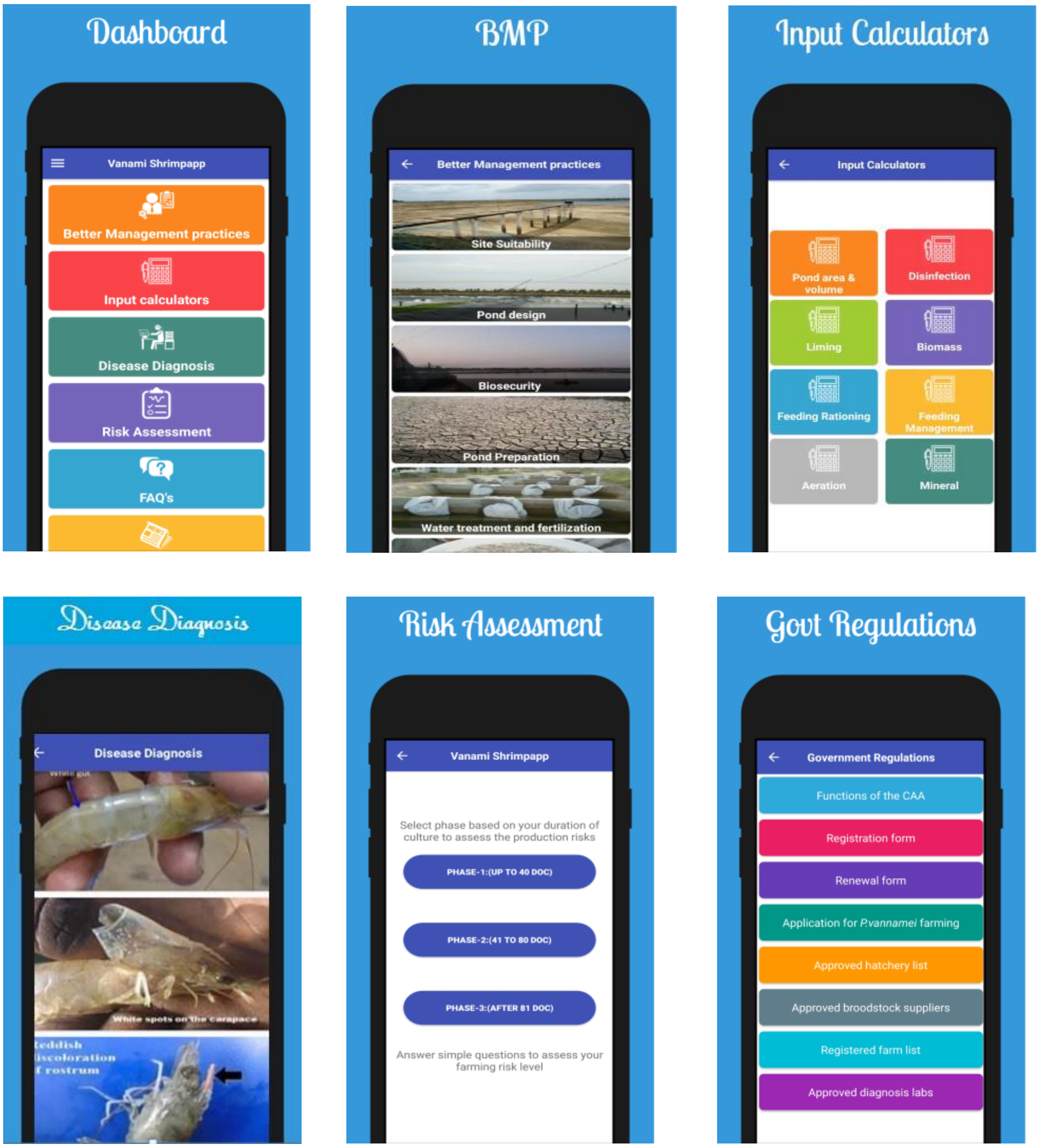
CIBA - Vanami Shrimpapp – the first mobile app on shrimp farming in India



Access to internet and smartphone penetration in rural areas, facilitated the use of mobile phones for technology communication and facilitates interaction among the research-extension-farmer-inputs-diagnostics-services and market. Shrimp farmers afford smartphones and Indian coastal states have adequate telecommunication infrastructure to access information through mobile phones. ICAR-CIBA has developed and launched an Android based mobile app – "*Vanami Shrimpapp*" for dissemination of technical information and interaction with the stakeholders of shrimp farming sector. The app was designed using android studio platform with SQLite databases. The app is available in Google playstore for free of cost and accessible offline mode too. Based on information need assessment study different static and dynamic modules with easy user interface were designed. Vanami Shrimpapp is the first mobile app on shrimp farming in India which provides technical support to the shrimp farmers, entrepreneurs and extension personnel and connects them with the scientific community.

BMP Module: Better Management Practices (BMPs) of shrimp farming which includes site selection, pond design, pond preparation, seed selection and stocking, feeding and feed management, soil and water quality management, health management, farming regulations, food safety and record keeping which are briefly explained with illustrations.

Input calculators: Vanami Shrimpapp contains various calculators pertaining to shrimp farming to estimate: the pond area and volume, total biomass in the pond, disinfection requirements, feed rationing, feed management, mineral requirement, soil pH adjustment and aeration requirement.



- **Disease Diagnosis (Probabilistic)**: Vanami Shrimpapp has a shrimp disease diagnosis module through which the user can diagnose the shrimp health and identify a probable disease by comparing the farm shrimp condition with list of images showing various primary and secondary symptoms. Upon selecting the relevant images, the module gives substantial information about the disease and enabling farmers to handle the situation wisely.

- **Shrimp farm risk assessment module:** This app has a farm risk assessment module which helps the user to evaluate the production risk status of his farm by answering a sequence of multiple choice questions. At the end of the module this tool will assess the potential risk level and recommend suitable management measures to manage those risk factors.
- **Update and advisories**: This app is supported with a dynamic module on advisories which enables the user to receive real time advisories, updates and market information.
- **Govt. guidelines and regulations**: The Government regulations and guidelines for shrimp farming are summarized in this module along with downloadable forms for registering farms with the Coastal Aquaculture Authority (CAA) and list of approved broodstock suppliers, hatcheries (seed sources), farms and laboratories (diagnostic labs).
- **FAQ Module**: Under the FAQ module one can find all possible queries along with explanations related to *Penaeus vannamei* shrimp farming. About 115 questions almost covering the total package of practices of shrimp farming were organized in to six major topics. User can change the language (vernacular) and font size to make it easier to read and understand. Keyword based search option is also available to list the queries on a particular topic.
- **Post a query**: This is the important feature of app through this the user can send his query and/or images of shrimp or pond and get an expert advice within two working days.

Downloads and performance of vanami shrimpapp

Vanami shrimpapp has been downloaded and regularly followed by more than 16000 stakeholders across the world. It has been extensively used by the farmers and extension workers across the countries viz. India, Indonesia, Thailand, Vietnam, Brazil, Peru, Mexico, Ecuador and USA (Fig.). The app has been appraised with a Google performance rating of 4.5 out of 5.0. The queries received through the app are answered within two working days. So far we have answered more than 3000 queries on vannamei shrimp farming through vanami shrimpapp asked by the farmers. This app has been developed based on the extensive farm surveys conducted with shrimp farmers of all the coastal states of India. However, the same content may be applicable to the vannamei farming in entire Southeast Asian countries and elsewhere in the tropical belts of the globe.

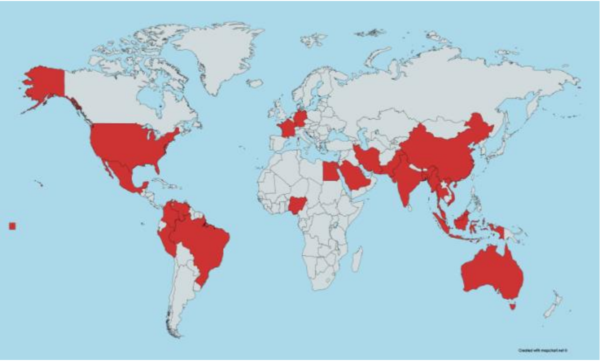


Fig. Usage of Vanami Shrimpapp countries

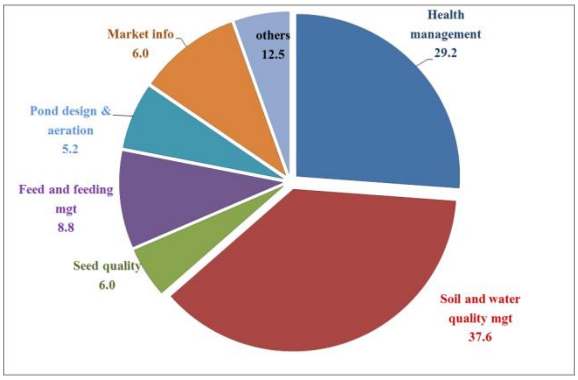


Fig. Subject matter wise queries received

Fig. Farmer consulting the Vanami Shrimpapp at his

Fig. Farmer after receiving response to his query through Vanami Shrimpapp mobile app

Conclusion

Stakeholders perceived that mobile phone based technology advisory could be the better option for capacity development and extension services in shrimp farming. Further updation of the mobile app with a facility to enter and upload day-to-day operations with real-time data analytics would aid in informed decision making at the farm level. This would subsequently lead to consolidation of data for supply chain integration and facilitate the evolution of next generation shrimp farming (shrimp farming 5.0) at the micro, meso and macro levels.

Economics of shrimp aquaculture in India

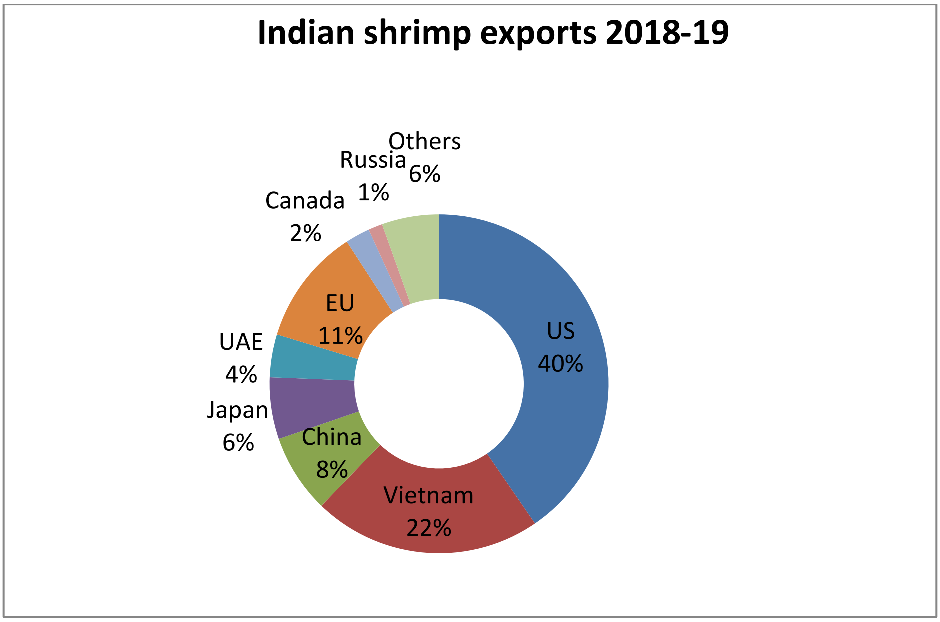
Ravisankar, T., Sairam, C.V and Geetha, R

India's exports of shrimp, the country's largest agricultural export item in 2018-19; surpassed 600,000 metric tons, in spite of unstable shrimp prices, dampening the export growth. Exports of the crustacean increased by eight per cent to 615,690t, according to International Trade Center, equivalent to some 44,495t more Indian shrimp this year compared to last year (Fig.1).

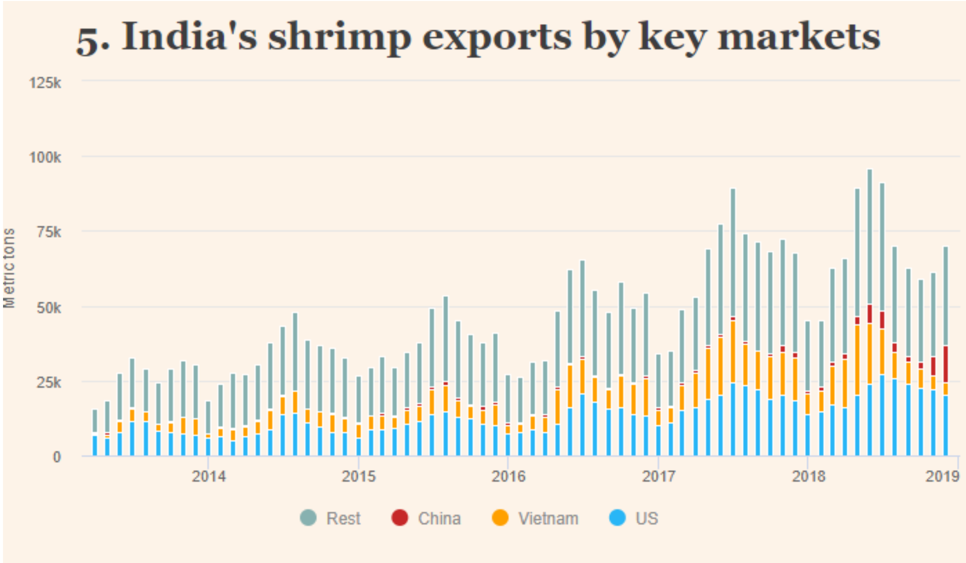
India, now the world's biggest exporter of shrimp, has been blamed for a crash in global shrimp prices which saw farm gate prices in some countries drop to decade-lows. Monthly average unit values of India's shrimp exports bottomed out last June at $6.92 per kilogram (Fig.4). After increase in July and August, unit values slipped again in December, falling to $7.32/kg. Lower shrimp prices may have checked India's export growth; 8% growth in 2018 was down from 31% growth in 2017. That year, India shipped an additional 134,889t to global markets (see chart two).

Exports to the EU declined 12% to 74,854t, while the total value of shrimp exports to the European Union fell 17% to $538m (Fig.3). Large volumes of Indian shrimp sent to the EU are processed in Vietnam, not included in these figures. Indian shrimp export growth was outpaced by Ecuador; the world's other main engine of farmed shrimp production growth.

But while Ecuadorian exports grew by 19% last year to 505,857t, India exports mainly de-headed and peeled shrimp, whereas Ecuador produces mainly head-on, unpeeled shrimp. By live weight, India produced closer to 750,000-800,000t, as per industry estimates. Hence, India's absolute growth by shrimp harvested was likely similar to that of Ecuador in 2018.



India is seeking other markets and may take confidence from a surge in exports to China in 2018-19, India exported 12,469t of shrimp to China, (Fig.5). The increase stems mostly from an increase in direct exports to China and decreases in shipments via Vietnam. The international trade arrangements are a matter of concern in agricultural exports and particularly in shrimp exports. It may be noted India has reached new markets like Middle East Asia and other nontraditional segments in the last decade. It is also imperative with as a large growing economy with increasing per capita incomes if domestic market is tapped India will have very less quantum of shrimp for exports as domestic prices are better than export price which is on the downslide.



The outbreak of early mortality syndrome, which struck traditional shrimp producing countries in China and Southeast Asia in 2012-13, was instrumental in driving expansion of India's shrimp industry though Indian farmers have also faced problems with EHP in shrimp farms. Farms expanded especially in eastern coastal states, such as Andhra Pradesh. Whatever may be the changes in global shrimp farming scenario India is a force to recon with by the shrimp producer and consumer nations.

Brackishwater Aquaculture for food, employment and prosperity

ICAR-Central Institute of Brackishwater Aquaculture

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