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## TRAINING MANUAL ON RECENT ADVANCES IN FARMING OF PACIFIC WHITE SHRIMP, *Penaeus vannamei*



**ICAR-Central Institute of Brackishwater Aquaculture**

Indian Council of Agricultural Research, Govt. of India

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ISO 9001:2015 certified



Training manual

# Recent advances in farming of Pacific White Shrimp (*Penaeus vannamei*)

**ICAR – Central Institute of Brackishwater Aquaculture**

75, Santhome High Road, R. A. Puram, Chennai – 600 028  
Tamil Nadu, India

Recent advances in farming of pacific white shrimp (*Penaeus vannamei*)

***Brackishwater aquaculture for food, employment and prosperity***

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# General

## **Shrimp farming in India: status and way forward**

Dr. K. K. Vijayan

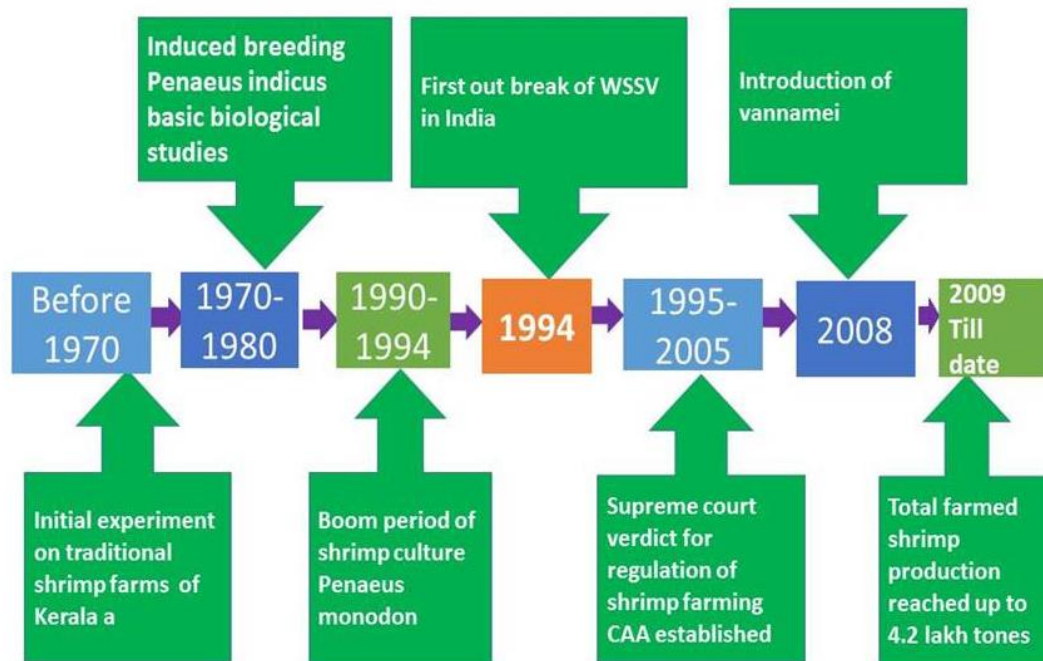
Shrimp is one of the most traded seafood commodities, and aquaculture of shrimp is considered to be one of the success stories of modern aquaculture. Shrimp farming generated millions of employments, and provides foreign exchange to developing countries. The growth of farmed shrimp production has been spectacular. Globally, farmed shrimp production grew from 10000 mt to 4 million mt. Indian shrimp farming sector also showed a remarkable growth, from 50000 mt in 1990 to 600000 mt in 2016. Although growth of shrimp aquaculture is remarkable, the sector has been facing several issues such as viral diseases, issues in marketing, and poor farm level performances. This article provides an over view of shrimp farming in India, and way forward for the long term sustainability of shrimp aquaculture.

### **Evolution of shrimp farming**

History of shrimp farming in India is almost similar to the other South East Asian countries. In early 1950s, juvenile shrimps were extensively fished from the paddy fields bordering the backwaters and estuaries of Kerala (pokkali), West Bengal (bheries), Karanataka (Ghazan) and Goa (Kazhan), and were exported to Myanmar to market as a shrimp product known as 'prawn-pulp'. Later at the advent of frozen shrimp industry in India, the demand for larger shrimps has increased considerably, and, therefore it was essential to grow the shrimp in the farm field to meet the demand of export industry. Thus the paddy field shrimp fishery has been evolved into a primitive form of aquaculture, where the naturally immigrating shrimp seeds from coastal waters are entrapped and prevented from returning to sea, and reared for few months, without any feed or aeration. Later, to augment the production, farmers started the practice of stocking the ponds with wild caught seeds, and thereafter, when commercial hatcheries started, with hatchery reared seeds. This form of improved extensive type of shrimp culture is still prevailing in Kerala with a production of about 400 kg/ha to 600kg/ha for a short period of culture without supplementary feeding, where it can be understood that this type of culture is a form of ecosystem based culture or an organic shrimp aquaculture, in perennial farms and pokkali rice farming fields.

Although extensive production system of shrimp started as early as 1960s, the industry only really began to intensify in the early 1990s, after the successful demonstration of commercial tiger shrimp hatchery in AP, through an MPEDA and DBT project, by TASPARG, with help of foreign technological support, which triggered the establishment of commercial hatcheries in private sector. However, this development has not happened in the already existing traditional shrimp farming regions: Kerala, West Bengal, Karnataka and Goa, and the modern shrimp aquaculture development largely centred in the areas where shrimp aquaculture did not have any prior history, such as Andhra Pradesh and Tamil Nadu. This can be attributed to the entrepreneurship of the local people, seasonal and geographical advantages. What followed is a spectacular growth of shrimp aquaculture system, during 1990-1995 with commercial hatcheries and farms with the use of desired seeds, formulated feeds and life supporting systems such as aerators. Farmed shrimp production showed a remarkable growth during this period of early 1990s, and thereafter production stagnated from 1996 to 2000, mainly due to WSSV pandemic, and related crop failures. Figure 1 provides the details of how shrimp farming in India has been evolved through the years.

## Time line on development of shrimp aquaculture

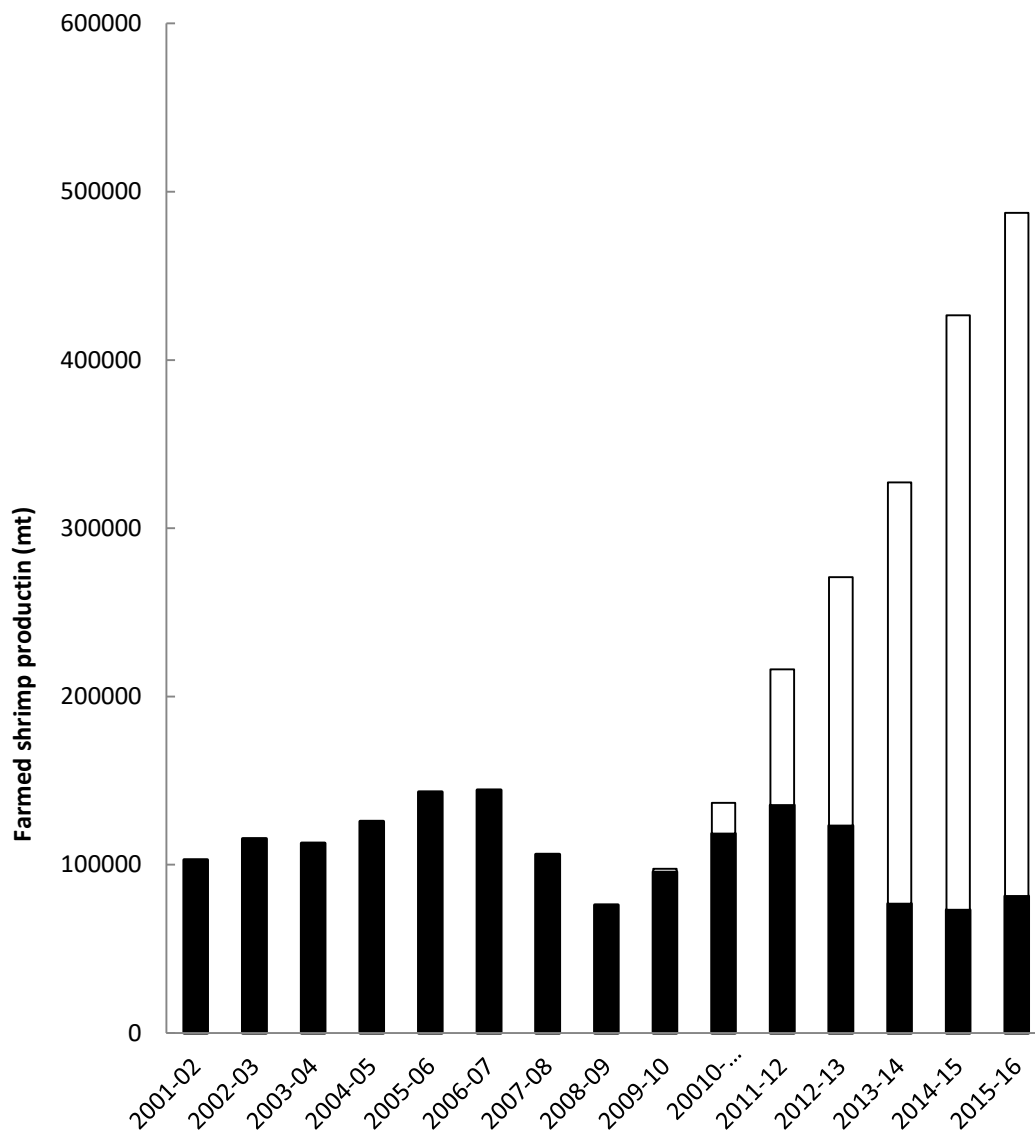


**Figure 1**

No therapeutic options available for the control of viral pandemics such as WSSV and the only management way out is to adopt preventive strategies. The use of post larvae generated from the specific pathogen free (SPF) broodstocks along with strict biosecurity measure are the most effective management option to ensure successful crops, Unfortunately, in India we did not have an SPF programme for any of the candidate species of Indian penaeids, the tiger shrimp or Indian white. Although development of SPF broodstock is time consuming and extremely difficult, it is essential pre-requisite for selective breeding. The US was successful in the selective breeding, which they initiate much earlier, resulted in the production of SPF *P. vannamei*, although the scale of shrimp farming was only limited in Americas. Again, the Taiwanese were the first to use SPF *P. vannamei* from US, with an initial success of *P. vannamei* production of 13 mt/ha within 75 days of culture. Following the success of Taiwan, *P. vannamei* was introduced into several South East Asian countries including India. In India, from 2010, a dramatic growth of farmed shrimp production due to the introduction of *P. vannamei* (Figure 2) was recorded, with 18247 mt in 2010 to 406018 mt in 2015-16. This was possible due to the superior aquaculture traits of *P. vannamei*, for example:

- Closed life cycle permits breeding and genetic selection program to be readily established
- Easier to cultivate at high density 60 to 150 PL/m<sup>2</sup>
- Can have good production up to 8 mt
- The wide range of salinity tolerance: from 0.5 to 45 ppt
- Highly tolerant to low temperature
- Low protein feed: 20 to 35%
- High survival rate in hatchery 50 -60%





**Figure 2: Farmed shrimp production in India,**

**Issues and challenges in shrimp farming**

The development of *P. vannamei* aquaculture has certainly not been without problems. Farming vannamei has been facing several problems in seed production, grow out and marketing.

**Problems in the seed production**

It has been noticed drastic decline in the productivity, in 2010 productivity per million seed was 21.4 mt, whereas in 2010 the productivity has reduced to 10.4 mt per million seed. These worrying downward trend cast doubt about the sustainability of farming of this exotic shrimp. Further, seed prices for vannamei in recent years have significantly dropped from 70 to 90 paisa/PL in 2012-14 to 30 paisa/PL. There are even reports that certain hatcheries are willing to supply seed at much lower prices. The high demand for vannamei has resulted in establishment of a large number of hatcheries. This has resulted in several hatcheries turning to pond reared brood stock rather than the imported SPF brood stocks. Use of pond

reared non SPF brood stock has resulted in inbreeding of the stocks and farmers currently receive non SPF seed with poor growth parameters. Larger farms have started to take seed from several hatcheries and rear them in separate ponds to identify the hatchery which is supplying the best quality seed. Again in this case, the marginal and small farmers are the worst affected class owing to their reduced buying power.

### **Higher cost of shrimp feed**

High cost of shrimp feed has been a major concern for shrimp farmers around the country. Most commercial feed manufacturers levy high rates for shrimp feeds thus seriously affecting the profitability of the venture. As on today, most commercial formulations charge around Rs. 72 to 75/Kg of shrimp feed. The prices of shrimp feed would be greater at distant locations like Haryana, wherein a new industry is shaping up. Shrimp feeds are sold at a higher price of Rs. 80 or more in Haryana. Small and marginal shrimp farmers with small holding sizes or leased land are the worst affected due to the increased shrimp feed prices. Such farmers are dependent on middlemen and have poor bargaining power compared to large farmers who directly source the feed from commercial feed manufacturers. It is estimated that the high cost of shrimp feeds have increased the production cost of farmed shrimp to about Rs. 220/kg.

### **Poor growth and stunting in vannamei**

Stunted growth of vannamei has been a major issue presently in the Indian shrimp farming sector. Most farms in Andhra have reported poor growth of vannamei. It has been observed that several farms reported a growth of 10 to 12 grams even after 110 days of culture. The exact reasons of stunting is not known, although researchers point towards the poor quality of seed and emerging diseases to be a major cause of the menace. Studies have shown that most hatcheries currently make use of pond reared broodstock which has the problem of inbreeding. Such inbred shrimp seed obviously will present a slower growth. Moreover, the greed to make more money has resulted in farmers skipping the basic pond preparation practices. Since, ponds are not dried between the crops and the soil not given sufficient time to release all accumulated organic matter, subsequent crops face production issues as a result of the reduced carrying capacity of the system.

### **Emerging diseases in shrimp farming**

Diseases have been a major cause for setbacks in shrimp farming. Several industry sources are of the opinion that the Indian farmed shrimp production set to decline in 2016 as a result of stunted growth and emerging diseases. White shrimp farmers across the country have faced the issue of new and emerging diseases most of which does not have an identified etiological agent. Some of the most reported diseases in vannamei farms in India for which a definite etiological agent have so far not been identified are Running Mortality Syndrome (RMS), Covert Mortality Disease (CMD), White Muscle Syndrome (WMS), bacterial white spots, White Gut Disease (WGD) and muscle cramping. One of the emerging diseases namely White Faecal Syndrome (WFS) often associated with poor growth of vannamei has been identified to be caused by a microsporidian parasite called Enterocytozoon hepatopenaei (EHP). Additionally, this year there has been a greater incidence of white spot virus (WSSV) and IHHNV outbreaks in several coastal districts of A.P. thereby seriously affecting the production in these areas.

### **Poor cooperation among farmers**

This has been a major cause of disease outbreaks in several areas. Certain farmers purposefully stock poor quality seed in high density to reduce production cost and finally end up having white spot infections. Such farms immediately practice distress harvesting by draining, thus resulting in disease spread in the whole area. The issues of common water intake and outlet have not been resolved in most farming areas.

### **Non adherence to regulations**

The coastal aquaculture authority (CAA) established under the Coastal aquaculture authority act of 2005 has laid down clear cut regulations for shrimp farming in the country which involves several means of ensuring a sustainable development of the industry. The registration of all coastal shrimp farms which has been a major objective and regulation under the CAA still continue to be a challenge for the authority and the nation. Even today, several farms in coastal areas do not have certificates of registration under CAA and several registered farms does not abide by the regulations made mandatory by the authority. One of the major reason for the issue is the poor staff strength of the authority which mostly depends on state and district level committees for its functioning. The authority has also laid down regulations for hatchery operation and farming for vannamei which are not followed. One of the major regulation of CAA, namely the effluent treatment pond (ETP) still continue to be distant dream for several farms thus seriously affecting the growth and sustainability of the industry.

### **The Way forward**

The introduction of *P. vannamei* has made appreciable changes in the production scenario of brackishwater shrimp culture in India. However, the development of *P. vannamei* aquaculture has certainly not been without problems. *P. vannamei* aquaculture in India has been facing several problems in the maturation and spawning (deterioration of male reproductive quality), in the larviculture (Zoea 2 syndrome) and in production system (early mortality syndrome and uncharacterized disease such as rapid mortality syndrome). The presumed inbreeding depression due to the large scale use of farm raised broodstock also reported to be one of the problems for the production losses. Further, broodstocks of *P. vannamei* is obtained from a single source, and this overdependence is found to be a distress to the further growth of shrimp industry in India. At this context, the development of native shrimp is found to be a viable option for the long-term sustainability of the industry.

### **Domestication and selective breeding of *P. indicus***

According to FAO data, about 600 species are being cultured world-wide; however, in reality the production is limited to 30 species. Introduction of species from one geographical area to another is common, and over 4000 introductions have been recorded in the FAO database for introductions. Although there are reports of few successful introductions without much recorded adverse effect, for example: introduction of GIFT strain of Nile Tilapia (*Oreochromis niloticus*) into many countries (Asian Development Bank 2005; De Silva et al., 2006), there are several documented cases of adverse environment effect of introduction of exotic species. Owing to the highly publicized incidents of escaped culture population, there are wide spread public concerns about the possibility of negative impact on native shrimp fauna. These impacts involve habitat destruction, introduction of pests and pathogens, completion of the feral exotic species with native species for food and space and displacement Therefore, many government agencies have taken keen interest in the

development of native species as signatory of biodiversity (Ross et al 2008). Further, even stake holders showed concern on overdependence on exotic *P. vannamei* culture in India. At this context, Indian white shrimp, *P. indicus* is found to be better alternative for the development of specific pathogen free stock for shrimp culture in India. The most important criteria for domestication and selective breeding of any species are complete control of reproduction under captivity. Although regulation of reproduction of penaeid shrimps has still been elusive goal of shrimp culturists, some penaeid species are relatively easy to breed under captivity, for example: *P. vannamei* and *P. indicus*. The relative ease of captive

**Culture performance of *Penaeus vannamei* (pre-domesticated) and *P. indicus***

Sl. No	Characteristics	<i>P. vannamei</i>	<i>P. indicus</i>
1	Pond size (ha)	0.1-0.5	0.6
2	Stocking density (shrimp/m <sup>2</sup> )	12	29.5
3	Initial mean weight (g)	0.01	<0.04
4	Final weight (g)	19.7	18.4
5	Days of culture	147	114
6	Daily weight gain(g/day)	0.13	0.16
7	Production (kg/ha)	2477	2557
8	FCR	2.1	1.6
9	Salinity (ppt)	28	11.1

breeding of *P. vannamei* has helped, to a large extent, in developing the domestication and selective breeding of *P. vannamei*. Indian white shrimp, *P. indicus*, is one of the first few penaeids whose breeding technology has been standardized. Further, initial experiments on the development of pond-reared broodstock also show the potential for development of domesticated stock for this species.

Growth and production performance are the important criteria for the candidate species for aquaculture. The tiger shrimp, *P. monodon*, received the high popularity due to its higher growth performance; this species attains 25-30 g within 120 to 130 days. The growth and production performance of *P. indicus* is comparable or even slightly better to the pre domesticated *P. vannamei* (Table). For example, *P. indicus* attained 18.4 g within 114 at a stocking density of 30 shrimps/m<sup>2</sup>, whereas *P. vannamei* took 147 days to reach similar body weight even at low stocking density of 12 shrimps/m<sup>2</sup>. Similarly the gross production was higher in the case of *P. indicus* (Table). More over this species is highly amenable to culture under high stocking densities and it has been reported a high production of about 16-18 mt/year in early 1990s.

**Potential advantages of developing selective bred *P. indicus* are multifold:**

1. As *P. indicus* are native species, all the quarantine measures to import *P. vannamei* could be avoided or minimize.
2. *P. indicus* is not a natural host of many emerging diseases, and it is comparatively easy to develop disease free stock.
3. In India, four distinct genetic populations of *P. indicus* have been recognized, and it indicates the potential for genetically distinct population.
4. As *P. indicus* is native to India it may exhibit greater tolerance and better growth than *P. vannamei*

5. This species is a strong osmoregulator and can cultivate under high saline and high temperature conditions

While formulating criteria for the development of native species for aquaculture Ross et al 2008 summarized basic requirements for establishment of aquaculture of native species. The success of domestication is largely depends on previous degree of domestication of this species and core scientific knowledge generated on this species. It includes: 1) Basic biology, 2) environmental physiology 3) closed reproductive cycle 4) nutrition 5) feeds and 7) on growing systems. The generation of this scientific knowledge is expensive and lengthy procedure. However, in the case of *P. indicus*, a large body of knowledge has already been developed in India.

In the last fifty years considerable advance has been made towards the successful domestication of *P. indicus*. Controlled of reproduction of this species have been extensively studied. Various aspects of reproductive endocrinology and vitellogenesis have been studied by and Diwan (1991a, b; 1992, 1993a, b, c; 1994) and Diwan and Mohamed (2007 a, b). Extensive studies on the basic biology of molting as well as molting as a function of growth have been studied by Vijayan and Diwan (1993, 1995, 1996 and 1997) and Diwan and Vijayan (2007). Nutritional requirements for *P. indicus* were studied by Gopal (1986). Ali (1982) and Vijayagopal et al (2008, 2009). The basic digestive physiology of *P. indicus* was studied by Hemambika (1989). Several on growing experiments on *P. indicus* have been conducted (Sivakami, 1988; Prasad, 1999). Thus, ample core science has been published, which is sufficient for the development of breeding program of Indian white shrimp. Initiation of ICAR-CIBA for the domestication of *Penaeus indicus*

Pilot scale farming demonstrations were conducted in all the coastal states of India to evaluate the production performance of native *P. indicus* at different agro-climatic zones. The production performance of *P. indicus* in terms of growth, productivity and disease occurrence were carried out in these trials. In all the demonstration trials post larvae produced by WSSV negative brood stocks were used. It is found that in most of the demonstration trials *P. indicus* performed par with the *P. vannamei*

## **Biology of Pacific white shrimp, *Penaeus vannamei***

Dr. Balasubramanian C. P., Dr. Kannappan S. & Dr. K. K. Vijayan

Aquaculture is the farming of fish, crustaceans, molluscs and aquatic plants in 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 rearing process, such as regular stocking, feeding or protection from the predators. Aquaculture is the fastest growing food producing industry with a total global aquaculture production of 73.8 million tonne. A total of 582 species are farmed worldwide and of these 62 are crustaceans. The total global farmed crustacean was 6.9 million tonnes valued for 37 billion USD. Although many crustaceans attract lucrative markets, shrimp has become the single most successful crops, 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 crops in coastal ponds/or coastal low lying ecosystems including India. The advent of sophisticated refrigeration facilities provided by artisanal farmers access by international markets. Thus traditional coastal aquaculture shifted to an export oriented or industrialized aquaculture. Farmed shrimp production has shown a 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 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 a basic biological knowledge with regard to the shrimp farming.

### **Nomenclature and Taxonomy**

**Shrimp versus prawns:** These two words are used synonymously in many literature, 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 difference between shrimp and prawns. However, presently at the Indian context we restrict to the consensus of FAO, and use 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 third but not with first. Females of this group release eggs directly to the water.

**Caridians:** Third pair of this group is not chelate, the pleura of the second abdomen overlaps first and third abdominal segment. Female carries the eggs until hatching

**Stenopodean shrimp:** Lesser known shrimp, third pereopod is chelate and considerably long, female carries eggs and pleura 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 are 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 argument for the reclassification of the genus *Penaeus*. However, the subsequent molecular studies carried out by researchers all over the world including researchers from CIBA, proved that no sufficient evidence for splitting the genus *Penaeus*. Currently all the 29 species is included in the genus *Penaeus*.

### ***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	<i>Penaeus</i>
Species	<i>Penaeus vannamei</i> (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 analog 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 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 symmetrical and semi-open. Spermatophores

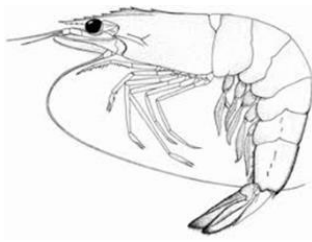


Figure 1 Penaeid shrimp

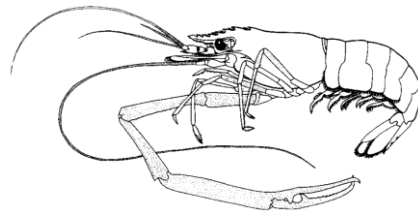


Figure 2 Caridean shrimp

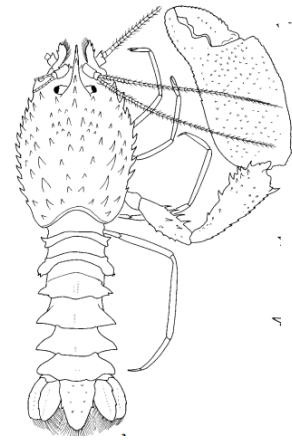
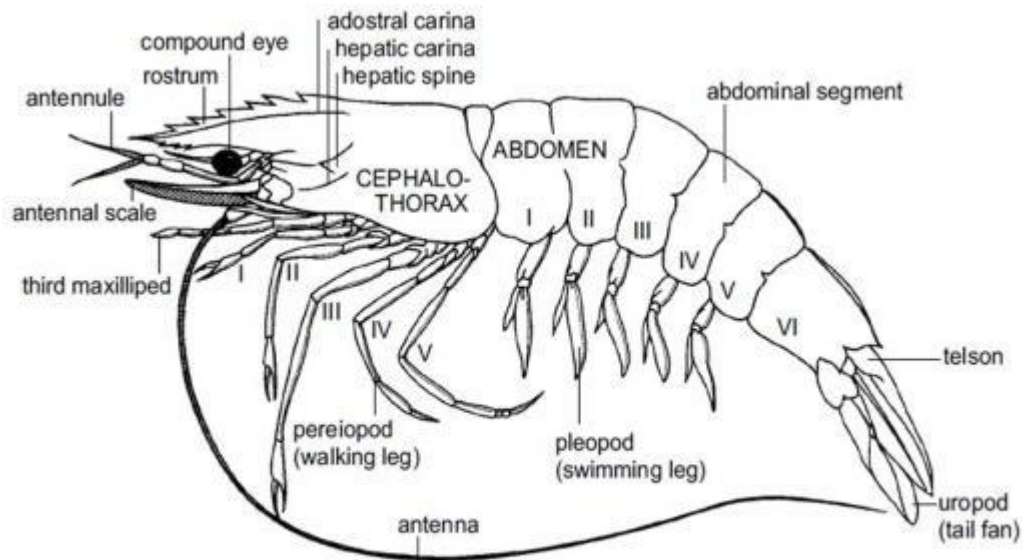


Figure 3 Stenopodean shrimp

**Figure three groups of shrimps: Penaeid, caridean and stenopodean shrimps**



**Figure of *Penaeus vannamei*: External morphology**

complex, consisting of sperm mass encapsulated by sheath. Mature female has open thelycum. Maximum size 23 cm, with maximum CL of 9 cm. Females 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 sexual 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 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, 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

### Life cycle of *P. vannamei*.

This species is distributed between Peru and Mexico, it prefers tropical marine habitats with temperature above 20° C. *Penaeus vannamei* shows 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 females of 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 estuarine environment has been made use of culture them in brackishwater ponds

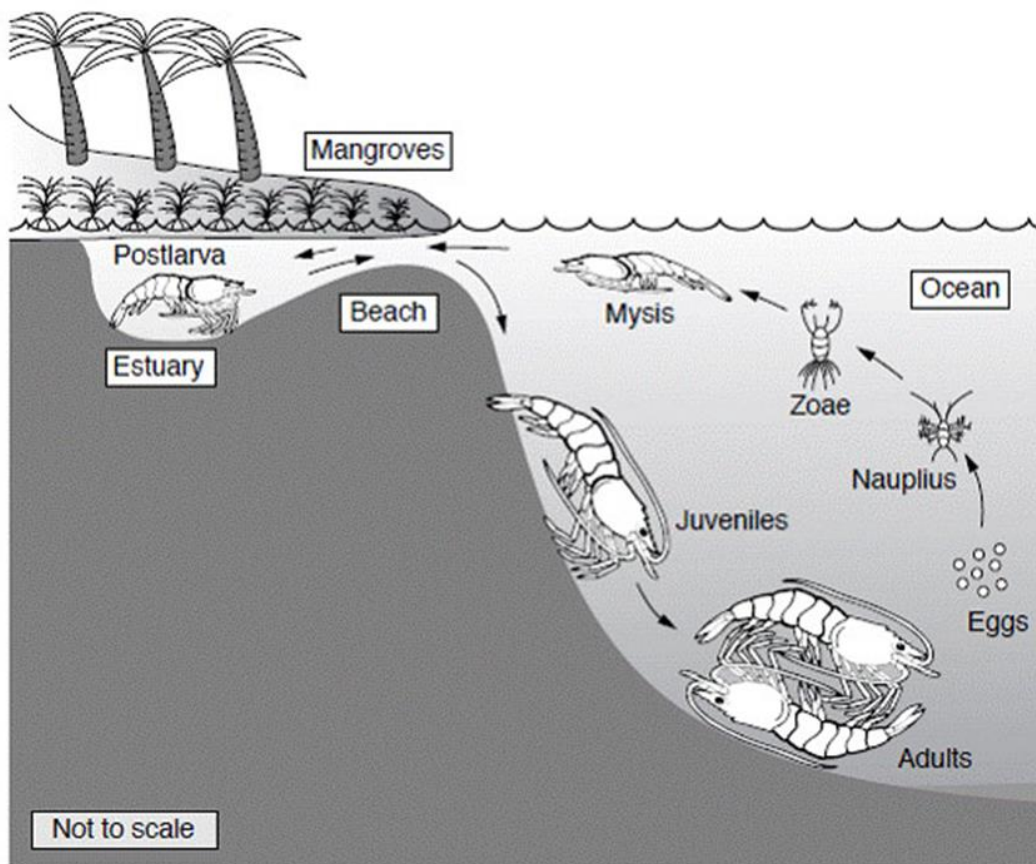
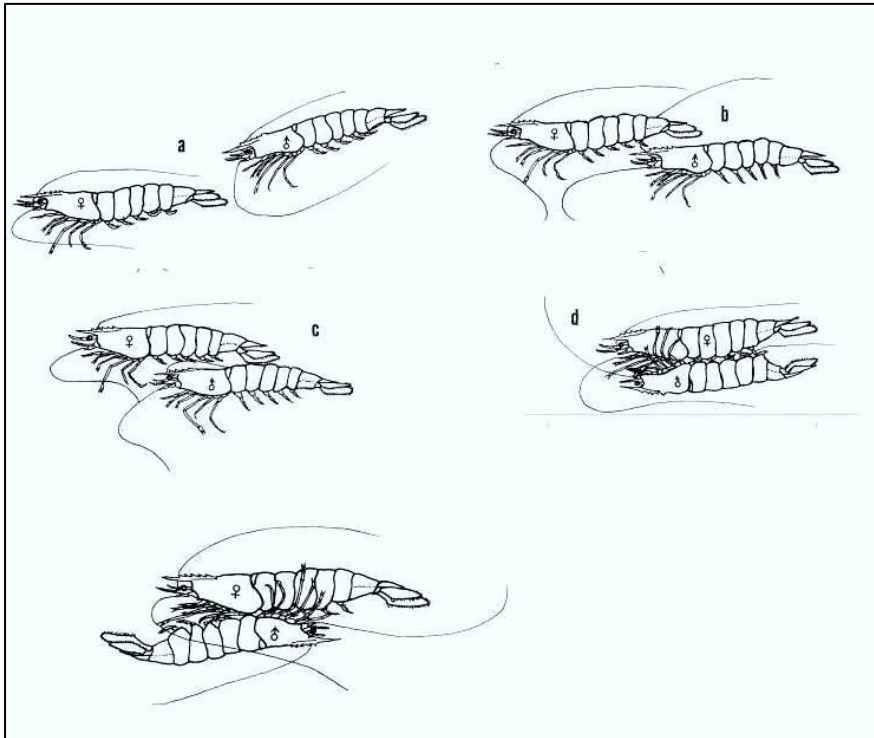


Figure 4. Penaeid life cycle (source: Rosenberry, 2009)



**Figure: Mating in *Penaeus 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 very early stage. Male possess a small rudimentary petasma, and thelycum of female is discernible as a small elevation between fourth and fifth pereopods. The males are generally mature at a smaller size than females. Male genital system comprises of 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, development of gametes (spermatozoa or sperms) is relatively straight forward without the intervention of 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, ovary grows in size and changes colour. This characteristic feature of vitellogenesis enables to classify the ovary into five maturation stages.

## **Hormonal control of reproduction**

Endocrinological/neuro-endocrinological studies on crustaceans started in first half of the 20th century. Generally, in invertebrates, oogenesis can be stimulated by a release from inhibition or by a 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. 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 eyestalk ablation procedure for inducement of reproductive maturation is successfully conducted by 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 early 1970s, this procedure has been continuing in commercial seed production industry across the world.

The most acknowledged consensus of crustacean reproductive endocrinology is that reproduction is controlled by two antagonistic hormones, 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 hydroxytryptamine), 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 has 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 the shrimp reproductive endocrinology.

The neural portion of the decapod eyestalk is an extension of 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 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 with typically 72 amino acids. Their protein precursor contains cryptic peptide, cHH precursor related peptide (CPRP), between signal peptide and cHH progenitor sequence. This type 1 cHH is named because upon injection it elicit 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 participates 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 crustacea. Gene coding for GIH has been characterized and cloned from several crustaceans: For example, terrestrial isopod (*Armadillidium vulgare*), lobsters (*Homarus americanus*, *H. gammarus* and *Nephrops norvegicus*) and shrimps (*P. monodon*, *Metapenaeus ensis*, *Litopenaeus vannamei*), prawn (*Macrobrachium nipponense*) and deep sea shrimp (*Rimicaris Kairei*). GIH is expressed in both male and female; it has been expressed in tissues such as eyestalk and brain. The presence of GIH has also found in larval crustacean as well. The expression of GIH mRNA is found to be lower in immature stage, however it was found to be higher in previtellogenic stage.

#### **Mating**

After sexual maturity, 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 ripe ovary, and towards the end of the molt cycle. Mating behaviour is triggered by photoperiod and occurs during sun set. 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 has 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 evolutionary old 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, Protozoa (=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 group this phase is embedded in the embryonic phase. The major morphological characteristic of this group is 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 swim 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 yolk stored in its body and moults five times before

metamorphosing into the next larval stage, the protozoa after 36-48 h. The protozoa are filter feeders feeding on the microalgae. There are three protozoal stage which 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 became carnivorous feeding on the small planktonic animals. The post larvae look like miniature of 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 morpho-physiological events. The most obvious manifestation of molt cycle is 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 early and 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.

# **Policies guidelines and energy use for sustainable aquaculture in India**

Dr. M. Jayanthi

Shrimp farming has grown rapidly in recent years in many tropical and subtropical countries, but there have been setbacks resulting from diseases and the growing awareness of 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 two and half 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 some environmental issues, and the need for regulatory mechanism to control the indiscriminate growth of aquaculture was realized.

## **1. Guidelines for aquaculture as per CAA act 2005.**

The Aquaculture Authority has brought out guidelines for the development of sustainable aquaculture. 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 metres 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 300m 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 metres 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 within 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 *L. vannamei* farms

- Aquaculture farmers who are registered with Coastal Aquaculture Authority will be required to submit a separate application for permission for farming *L. vannamei*. In case of so far unregistered farms, the application for registration must clearly spell out the intention to culture *L. 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 *L. vannamei* applications shall be processed by the Member Secretary for consideration of the Coastal Aquaculture Authority for issuing permission to farms for farming of *L. 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.
- *L. 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 *L. vannamei* in fresh water / inland farms

Government of India have communicated approved guidelines that the farmers who desire to culture the exotic species, *L. 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 *L. 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 *L. 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 *L. vannamei* in fresh water / inland farms located outside the jurisdiction of CAA.



- Permission for taking up culture of *L. 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 *L. 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 *L. 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 *L. 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 *L. 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 *L. 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. Guidelines for hatchery**

#### **3.1. Criteria for application to breed SPF *L. vannamei*.**

(1) Hatcheries engaged or intending to be engaged in shrimp seed production having the required bio-security facilities as prescribed by Coastal Aquaculture Authority would be eligible to apply for registration under the Coastal Aquaculture Authority Act, 2005 and the Rules framed there under and for permission to import SPF *L. vannamei* broodstock and to produce and sell post larvae of *L. vannamei*.

(2) Approval of the hatchery for rearing *L. vannamei* will be given by Coastal Aquaculture Authority after due inspection of the hatchery facilities by a team constituted by Coastal Aquaculture Authority for this purpose.

(3) The hatchery facilities should have strict bio-security control through physical separation or isolation of the different production facilities which is a feature of good hatchery design. In existing hatcheries with no physical separation, effective isolation may also be achieved through the construction of barriers and implementation of process and product flow controls.

(4) The hatchery facility should have a wall or fence around the periphery of the premises, with adequate height to prevent the entry of animals and unauthorized persons. This will help to reduce the risk of pathogen introduction by this route, as well as improve overall security.

#### **3.2. Sanitary requirement**

(1) Entrance to the hatchery should be restricted to the personnel assigned to work exclusively in this area and a record of personnel entering the facility should be maintained by the security personnel.

(2) Hatchery staff should enter through a shower or dressing room, where they remove their street clothes and take a shower before entering another dressing room to put on working clothes and boots. At the end of the working shift, the sequence should be reversed.

(3) There should be means provided for disinfection of vehicle tyres (tyre baths at the gate), 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.

#### **3.3 Water intake**

(1) Each functional unit of the hatchery should have independent water treatment facility and it should be isolated from all other water supply systems. Separate recirculation systems may be used for each functional unit of hatchery to reduce water usage and improve bio-security, especially in high-risk areas.

(2) Water for the hatchery should be filtered and treated to prevent the entry of vectors and pathogens that may be present in the source water. This may be achieved by initial filtering through sub-sand well points, sand filters (gravity or pressure), or mesh bag filters into the first reservoir or settling tank. Following primary disinfection by chlorination, and after settlement, the water should be filtered again with a finer filter and then disinfected using ultraviolet light (UV) and/or ozone.

(3) The water supply system may include use of activated carbon filters, the addition of ethylene diamine tetra acetic acid (EDTA) and temperature and salinity regulation.

### **3.4. Water treatment and discharge of waste water**

(1) The discharged water from the hatchery should be held temporarily and treated with hypochlorite solution (>20 ppm active chlorine for not less than 60 min) or other effective disinfectant prior to discharge. This is particularly crucial where the water is to be discharged to the same location as the abstraction point.

(2) The seawater to be used in the facility must be delivered into a storage tank where it will be treated with hypochlorite solution (20 ppm active ingredient for not less than 30 minutes) followed by sodium thiosulphate (1 ppm for every ppm of residual chlorine) and strong aeration.

(3) No waste water shall be released out of the hatchery without chlorination and dechlorination, especially to prevent the escape of the larvae into the natural waters. Effluent Treatment System (ETS) should be designed to include this provision.

### **3.5. Disinfection of implements**

(1) Used containers and hoses must be washed and disinfected with hypochlorite solution (20 ppm) before further use.

(2) Each brood stock holding tank should have a separate set of implements which must be clearly marked and placed near the tanks. Facilities for disinfection of all the implements at the end of each day's use should be available.

### **3.6. Brood stock in hatchery**

(1) Only SPF brood stock cleared through the quarantine should be used in the hatchery for seed production.

(2) Use of pond-reared brood stock is strictly prohibited.

(3) Hatcheries involved in *L. vannamei* seed production should not use any other species within the hatchery premises.

## **4. Aeration requirements**

Aeration of shrimp pond water has become an essential requirement for the culture of *L. vannamei* to maintain the required dissolved oxygen level and to keep the water in circulation. The shrimp farmers use different types of paddle wheel aerators with custom made design or other types of aerators such as diffuser type or submersible type. Optimization of aerator use is very essential since too little aeration could lead to hypoxic conditions in ponds resulting in mass mortalities and excess aeration could result in higher operating expenses and wastage of energy. At present, shrimp farms produce 5 to 9 tonnes per ha in *L. vannamei* farms compared to average of one to two tonnes per ha production in *P. monodon* farms in the country. The aeration levels have a strong impact on the growth of *L. vannamei* and normally one HP aerator is used for 300 to 400 kg of biomass, which is much higher level than the actual requirements. Cost of electricity / diesel for aeration is the second highest expenditure after feed and account to 15-30 % depends on the management practices followed in the farms. Too little aeration lead to hypoxic condition in ponds thereby affecting the growth and survival of the shrimps and high level of aeration results in excessive operating expenses and wastage of energy. The first study by CIBA with NFDB

funding support was executed to assess the prevailing status of energy use in shrimp farms, indicated the vast variation in energy use among the farmers due to fear and unawareness. It is suggested to maintain the DO requirement based on the necessity to save the production cost and energy use.

### **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. Self-discipline is the secret of sustainability. Therefore, 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.



# **Seed production**

## Broodstock development and Maturation of *Penaeus vannamei*

Dr. Shyne Anand P.S. and Dr. C. P. Balasubramanian

### 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 its reproductive performance. Expansion of *P. vannamei* farming across the world can be credited to fast growing and disease resistant strains developed through selective breeding programs. This species can be readily reproduced in captivity, has wide tolerance to environmental parameters, better utilizes low-protein containing diets, and grows fast compared to other penaeid shrimp species (Wyban, 2007).

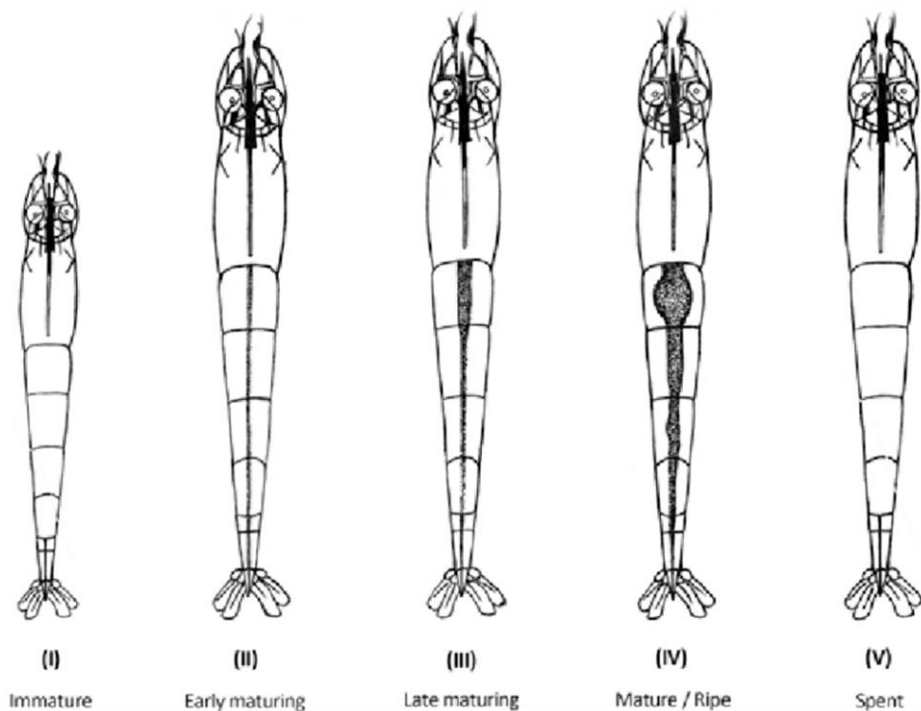
Like any other marine penaeid shrimps, adult *P. vannamei* lives and spawns in sea. The larvae undergo metamorphosis in sea and post larvae migrate to brackishwater environments while juvenile and sub adult spends life in coastal estuaries or lagoons. It is reported that in nature male mature at 20g size and female at 28 g size. However, in hatchery broodstock weighs 40-45 g size is preferred. Female reaches sexual maturity 8-10 month while male reaches above 10 month. *P. vannamei* is an open thelycum species. It mates when both male and female are in hard stage and after mating spermatophore can be seen as white sperm plug glued to thelycum.



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

**Stages of ovary development:** 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 or post spawn



## **Induced maturation techniques**

### **Eye stalk 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 2/3<sup>rd</sup> of the eye stalk 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, moulting, carbohydrate, protein or lipid metabolism etc. Removal of one eye stalk reduces gonad inhibiting hormone level produced by the XO-SG in 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. 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. Similarly, progesterone was also found to influence ovarian development and spawning and



improve sperm quality in *P. vannamei*. Recently, it has been reported that GnRH plays an important role in the development of ovarium in *P. monodon*.

### **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 important for reproduction. Ideal reproductive environment is utmost important for shrimp maturation. Environmental parameters play an important role in maturation. Temperature generally keep at 27-29°C and male brooder tanks need comparatively lower temperature to maintain sperm quality, 26-27°C for gonad maturation through seawater chillers while female brooders keep at 28-29°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 maturation room.

### **Live food and feed management**

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 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, 20:4 $\omega$ 6), eicosapentaenoic acid (EPA, 20:5 $\omega$ 3) and docosahexaenoic acid (DHA, 22:6 $\omega$ 3) which are essential for shrimp maturation. At present, shrimp industry uses 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 hormonal active substances. Feeding is generally provided at 25% and recently increased upto 50% of body weight.

### **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<sup>2</sup> if artificial insemination procedure is followed. Generally, it is reported that stoking density must be 0.2-0.3 kg per m<sup>2</sup> based on biomass. Segregation of sex and stocking in different tanks are practiced for fed manipulation, sperm quality improvement like maintain temperature at 26-27°C temperature, artificial insemination etc. Artificial insemination is also followed spermatophore from male is removed by pressing the 5<sup>th</sup> 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.

## 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 be spawn about 10-12 times based the amount of quality maturation diet. Individual spawning is preferred to reduce horizontal transmission of diseases though majority of the hatcheries follows 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 (2<sup>nd</sup> 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 scoopnet. The tank water was drained and the eggs were passed through a 350 micron hand net which retains faeces 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 heavy metal load in water. Periodic shuffling is done to improve hatchability.

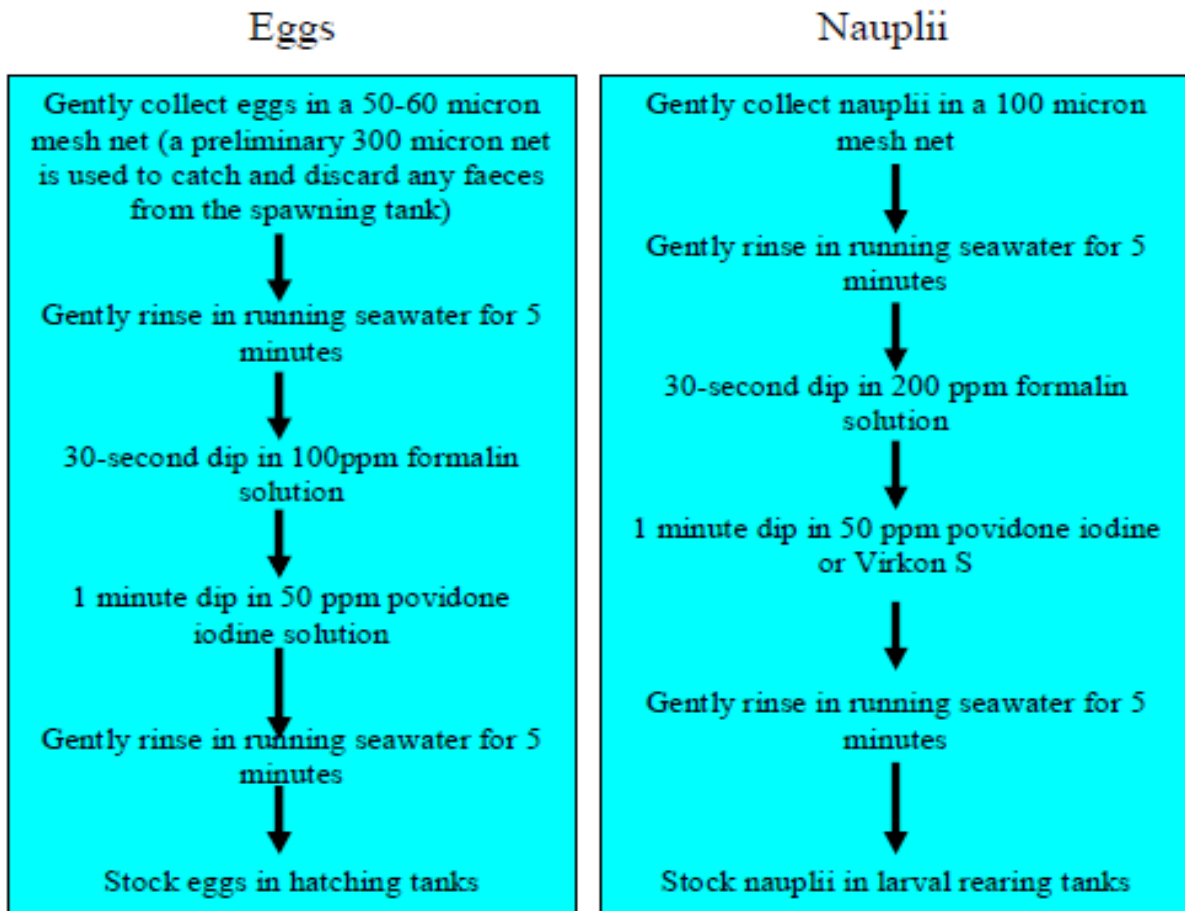
$$H\% = \frac{Y}{X * 100\%}$$

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



### Further reading

1. Wyban, J., 2009. World shrimp farming revolution: industry impact of domestication, breeding and widespread use of specific pathogen free *Penaeus vannamei*. In: Browdy, C.L., Jory, D.E. (Eds.), *The Rising Tide—Proceedings of the Special Session on Sustainable Shrimp Farming*. The World Aquaculture Society, Baton Rouge Louisiana, pp. 12–21.
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4. A. P. Dineshbabu Geetha Sasikumar Prathibha Rohit Sujitha Thomas K.M. Rajesh P.U. Zacharia 2014. *Methodologies for studying fish and shell fishes*. Central Marine Fisheries Research Institute (Indian Council of Agricultural Research). 46pp

## Concept of SPF, SPR, BMC, NBC in genetic improvement programs

Dr. Vinay T. N., Dr. Shyne Anand and Dr. Sudheer N. S.

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?***

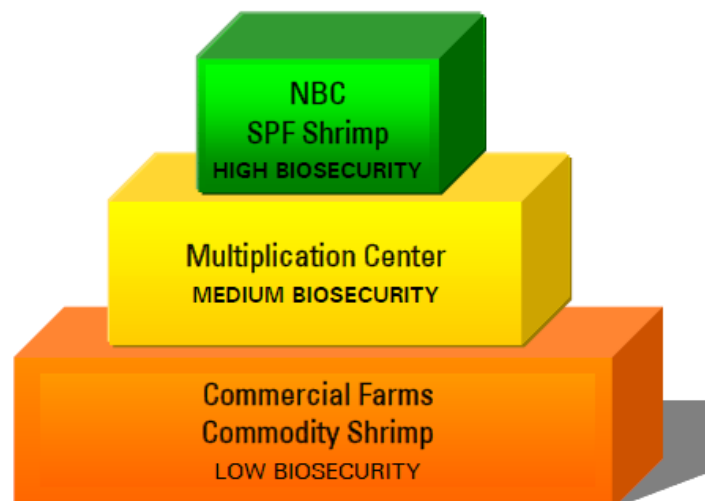
The concepts represented by the acronyms SPF, SPR and SPT have been used in a rather confusing manner over the last few years. SPF refers to the sanitary status of a stock, which means the stocks are free of certain pathogens, but not free from all pathogens, and not simply PCR negative. SPF stocks are 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.



Pictorial representation of a genetic breeding program facility

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.

**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. <http://dx.doi.org/10.4236/as.2014.512125>

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.

## Larval rearing protocols for *Penaeus vannamei*

Biju I.F., Dr. Shyne Anand, Aravind R & Jose Antony

### 1. Harvest and stocking of Nauplii

Shrimp eggs hatch out in to nauplii stage after 18-20hrs of incubation. Throughout the larval rearing period, animals must not be exposed to abrupt changes in environmental conditions. Instead they should be given time to gradually adapt to new conditions to avoid stress and mortalities. Once the eggs hatch out, the nauplii can be seen in the water by examining in the beaker. When the hatching completes they must be harvested and kept in the larval rearing tanks for further rearing up to post larvae stage which eventually becomes the stocking material for shrimp farming. The procedure for the collection and stocking of the shrimp larvae is given below.

- Turn of the aeration in the hatching tank and cover it leaving a small portion for entry of light. Being attracted to light the nauplii will concentrate on this area. With the help of a small hose pipe, the nauplii can be siphoned out into a nauplii harvesting bucket. The nauplii harvesting bucket is fitted with a spout of 100 micron net to remove excess water.
- The healthy nauplii concentrate at the light within 30 minutes once the tank is covered. They are the healthy larvae which can be harvested with in the first siphoning attempt. The remaining nauplii which does not come light source are weak and can be discarded.
- After concentrating the nauplii in the collection container they can be counted for estimating the total nauplii count. This is necessary for stocking the nauplii in the correct density in larval rearing tanks. As well as, the various larval feed requirements is also determined according to the nauplii count.
- For taking the nauplii count, take out 3 samples of 100ml and count the number of nauplii present in each subsample. Take the average of the samples counted and multiply by 10 to determine the count in one liter. Estimate the volume of the nauplii collecting container and find out the total count of nauplii present in the container.
- For stocking the nauplii, make ready the larval rearing tanks and fill with fresh filtered sea water. Follow the method for calculating the volume of water to be taken from the nauplii collecting container for stocking.

$$V = \frac{S \times L}{D}$$

S= Stocking density in Larval tank (100 nauplii/litre)

V= Volume of water to be taken from the nauplii collecting container (litres)

D= Density of nauplii in the collection container (No/litre)

L= Volume of water in the larval rearing tank (In litres).

In commercial shrimp hatcheries for conveniently doing the above procedure the nauplii collected after spawning or sourced from other certified *vannamei* hatcheries are concentrated into a 100 litre bin with filtered seawater and aeration. From this bin a sample is taken for counting. Usually the sample volume taken is 1 or 2ml; this is done because larger volumes will be difficult to count because of the high density of the larvae in the tank. The counting is done three times and the average is taken. From this figure the total count of nauplii present in the tank is estimated. After the counting procedure the larvae is given

prophylactic treatment with formalin and treflan. 1ml of formalin is added to 10 litres of water and the nauplii is dipped in the solution for 20 seconds and rewashed in seawater. Similar procedure is followed for the treflan treatment also. These treatments are given to the larvae to disinfect them from fungal and bacterial attack.

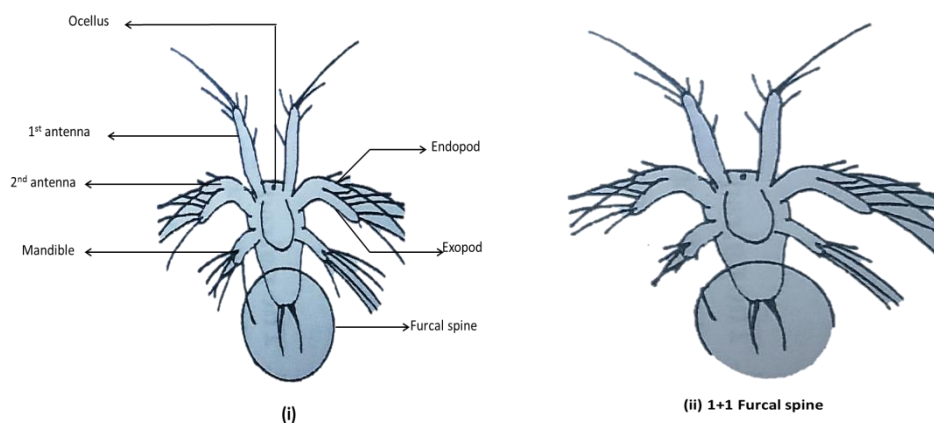
Shrimp hatcheries have larval rearing tanks (LRT) of 10-12 tonne capacity. For stocking the nauplii stage the tanks are partially filled with filtered seawater. The initial stocking density is maintained at the rate of 300-400 larvae/litre. The initial water level is kept lower because the microalgal feed will be pumped daily to the larval rearing tank for feeding the larvae and this will raise the water level in the tanks.

The shrimp larval cycle pass through 12 stages before getting converted into post larvae. As the larvae pass through different stages their behavior changes and needs special care in each stage. For successful rearing of the shrimp larvae identification of the larval stages is very important.

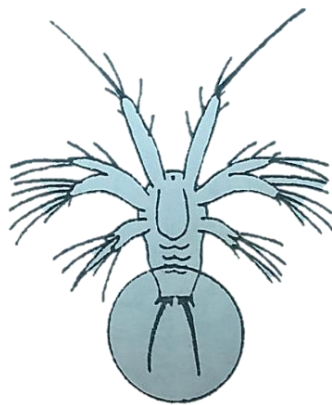
## 2. Shrimp larval stages

### Nauplius stage

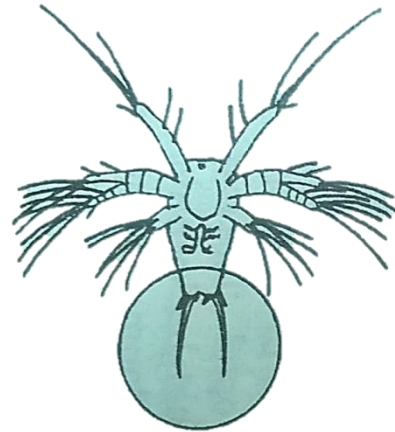
The nauplius stage is the first stage which comes out of the hatching eggs. They are very tiny measuring from 0.30 to 0.58 mm in total length. This stage is attracted to light and in aerated tanks they will concentrate in most lighted area when aeration stops. The nauplius stage comprises of six sub 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.



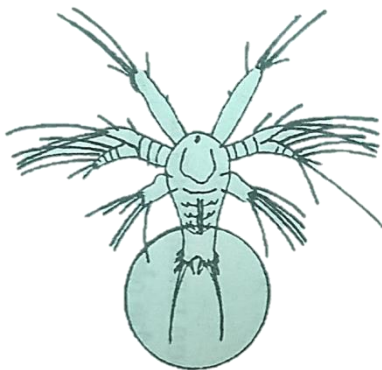




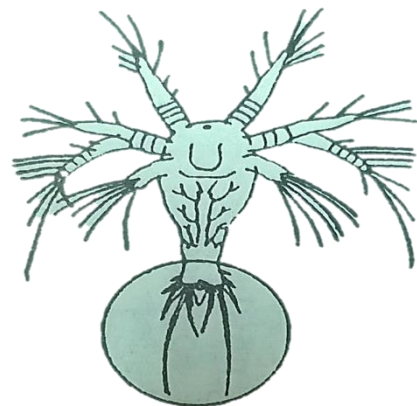
(iii) 3+3 or 2+2 Furcal spine



(iv) 3+3 or 4+4 Furcal spine



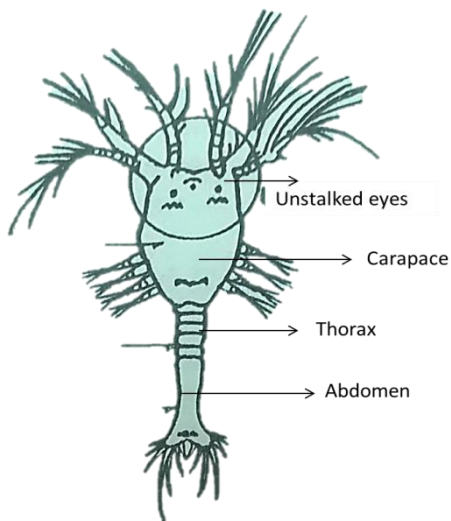
(v) 5+6 or 5+5 Furcal spine



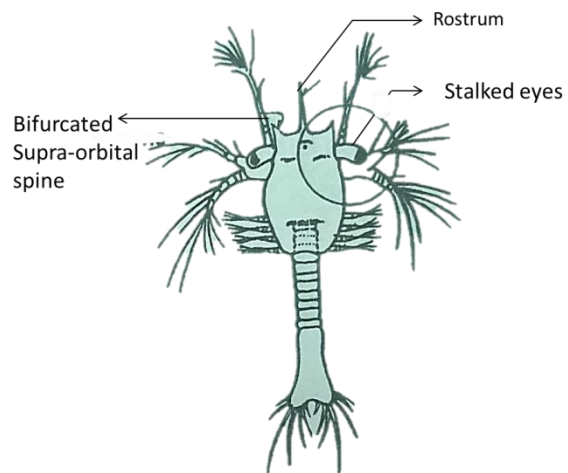
(vi) 6+7 or 7+7 Furcal spine

### Protozoa stage

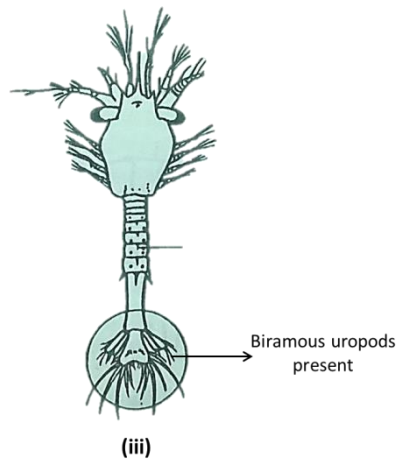
The protozoa can be easily identified from the nauplius. It consists of a carapace, thorax and abdomen. The paired eyes can be seen on the protozoa stage. The protozoa stage also undergoes through three sub stages viz. protozoa 1, 2 and 3. The sub stages of the Zoea can be easily identified under the microscope. The eyes become stalked and the presence of rostrum marks the zoea 2 stage. In the zoea 3 stage the biramous uropods become visible.



(i)

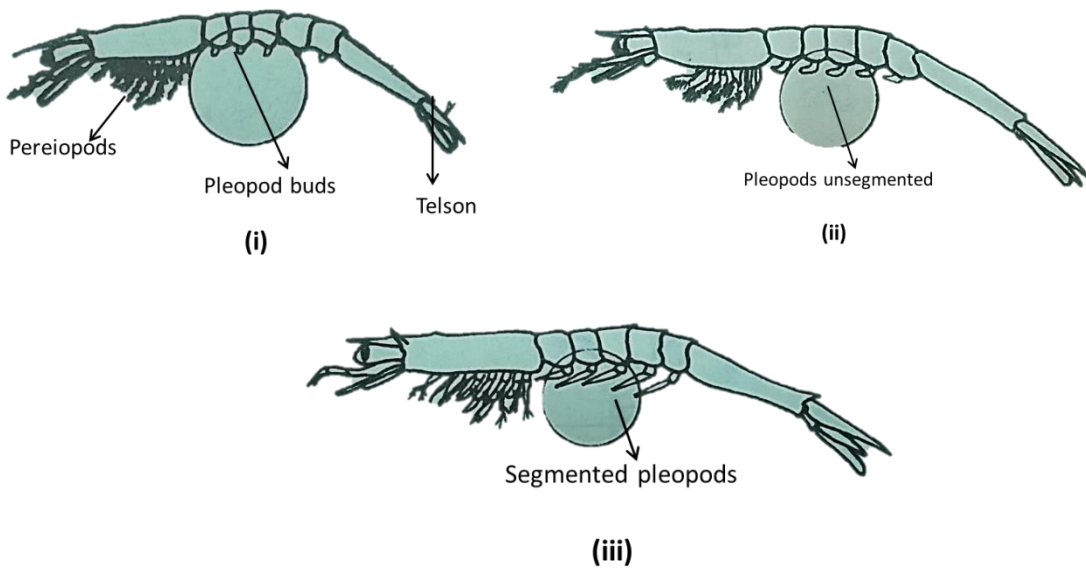


(ii)



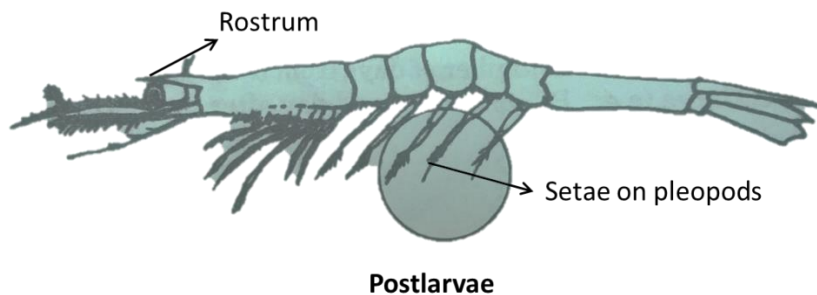
Mysis stage

The mysis stage has shrimp like appearance and its head points downward. Its body measures from 3.28 to 4.87 mm in total length. Mysis stage also comprises of three sub stages. The mysis stages are identified by the presence of pleopod buds. The pleopods appear as buds in the mysis 1 stage, which gets protruded at mysis 2 stage. In the 3<sup>rd</sup> stage, the pleopods become segmented and can be identified by observing under microscope.



Postlarval stage

The postlarva resembles an adult prawn. At postlarval stage the rostrum gets extended and exceeds the tip of the eye and setae will be clearly visible on the pleopods.



### 3. Feeding the shrimp larvae

The initial feeding of the larvae is done using microalgae of suitable cell density. For feeding the protozoa stage 50,000 cells/ml algal density is maintained. Diatoms such as *Cheatoceeros* and *Skeletonema* can be used. The microalgal culture in the outdoor algal culture tanks with suitable cell density are pumped into the larval rearing tanks. Algal culture from outdoor mass culture tank is pumped into the larval rearing tank @ 200litre during in the start of zoea stage. Adequate algal density is maintained in the tanks by periodic examination of the larvae and water in the tanks. Using a beaker of 250 ml capacity samples are taken from the tanks for observation of the larvae and algal content. For maintaining hygiene, one sample beaker per tank is kept in a 5 litre bucket with formalin solution to avoid the cross contamination. The sample is taken periodically to understand the larval metamorphosis and feed intake. According to these observation fresh algae is added into the tanks if needed. If the zoea stage is healthy and actively feeding trailing fecal matter can be seen from the larvae. Ribbon like fecal matter can be seen in the water if the zoea stage is properly taking the feed. If the larva is actively feeding 500litre of algal culture is pumped in the morning and evening/day/tank.

The zoeal stage undergoes moulting and passes through three zoeal stages viz Z1, Z2 and Z3. Normally one zoeal stage takes 1 to 2 days to transform to the next stage. Usually the zoea stage takes around 4-6 days to complete the zoeal stage and transform to mysis stage. During the whole zoeal stage microalgae is the major feed item. From zoea stage two onwards micro coated feeds of size 50-60 micron is provided along with the algae. This micro coated feed is usually provided @ 20 gm for 1 million larvae/day at three or four times daily.

As the larval stage proceeds, approximately after 4-6 days mysis stage begins depending upon the temperature. Mysis stage also comprises three levels with metamorphosis on each day. Feeding schedule also changes along with this change in larval stage. Microalgal feed is given as in the previous stages. The water level in the tank also rises in this phase as microalgal feed is pumped into the tanks. If needed fresh seawater is also provided. As the larvae changes in size the micro coated feed size also can be increased. In this stage 100-150 micron feed is given as complementary feed along with the algae. Late mysis or PL stage onwards live *Artemia* nauplius can be given at 5 nos. /PL.

Within 3-4 four days the mysis stage transforms into the post larvae (PL1) stage. Microalgal feed at lower densities can be provided in postlarval stages. Post larval stages are reared using micro feeds and artemia. The amount of artemia cyst required for 1million PL is 100gm/day. Water exchange is done during this stage of the larvae. About 50 % of the water is exchanged using a siphon pipe with suitable mesh screen. The debris inside the tank is also removed by a siphon. For this purpose the larvae is allowed to surface by switching of the aeration. Micro feeds of the size 200 micron are provided from PL3 stage onwards. When the Post larvae reach the PL15 stage, they are ready for packing and stocking in the farmer's pond. Salinity reduction should commence only when PL reaches 10 or above when gills are fully developed. Salinity reduction must be done 3 ppt per hour from 30-20 ppt; 1 ppt per hour from 20 ppt to 10 ppt and 0.5 ppt per hour from 10 to 5 ppt.

#### **4. Larval quality assessment**

Microscopic examination of larvae for analyzing the PL quality should be done periodically. Necrosis, deformities, epibiont fouling, muscle: gut ratio, squash mount, staining and basic bacteriology can be done as a routine. PCR screening of the postlarvae for viral diseases like WSSV, IHHNV is a must before handing over the seed to farmers. Every hatchery shall have in house PCR facility and qualified personnel for routine testing of the postlarvae against the viral diseases.

#### **5. Packing and transport of shrimp seed**

Shrimp seeds at the stage PL13 and above should be selected for packing. Before packing the seed the salinity at farmer's pond should be checked or enquired one day in advance so that the larvae can be acclimatized to such salinity levels without any stress. On the day of transportation, the PLs should be carefully harvested from the larval rearing tanks with the help of a PL scoop net. The larvae thus collected should be kept in cooled sea water with ice bags. Decreasing the temperature from 28-30 to 23°C should take place gradually. In the mean while packing bags can be kept ready by filling water. Polythene bags are used to pack the shrimp larvae. Usually it is a double bag package with an inner polythene bag where larvae is placed and an outer cover for support. The number of larvae stocked in the bag depends upon the time taken to reach the destination. Post Larvae 12-15 can be transported at 2000 nos. per pack in air conditioned vehicle. If more time is required to reach the destination the number of larvae in each bag is reduced. The polythene bags are filled 1/3<sup>rd</sup> with sea water and remaining part oxygen. Packed ice bags should be provided to maintain the temperature during transport. Live artemia nauplius need to be added at 20 nos. /ml as live feed during transportation.



# Live feed

## **Live feeds for brackish water Aquaculture**

Aravind R, Sandeep K P, Jose Antony, Biju I F, Dr. Shyne Anand, Dr. S. Kannappan & Dr. S. Sivagnanam

Intensive larviculture of fin fish and shell fish species has been expanded into a multimillion dollar industry over the past 2-3 decades. Mass scale culture of early larval stages requires use of live feeds as their dietary requirements. Live feeds are essentially any feed which is not processed to a significant degree. Live feeds are usually fed as alive or a whole but may be chopped up, cooked, frozen or blanched. 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 convenience 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: Importance in aquaculture**

Marine 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.

### **Culture techniques**

Microalgae can be produced using a wide variety of methods, ranging from closely-controlled laboratory methods to less predictable methods in outdoor tanks. Various chemical media are 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°C), CO<sub>2</sub> (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).

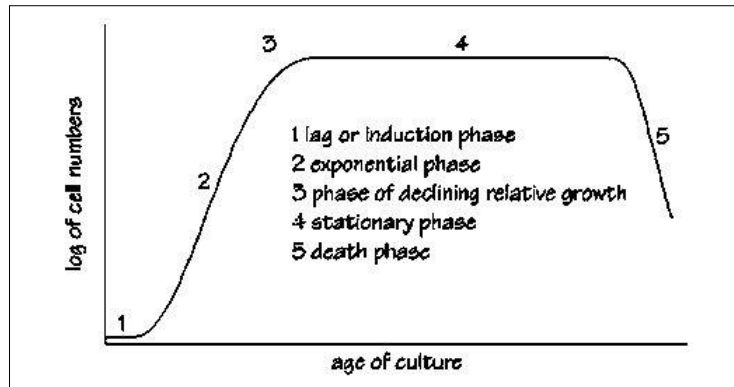


Fig1. Growth phases in the microalgal life cycle

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 laboratory

- ✓ **Axenic (=sterile)/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 ml flasks, 3 and 20 l carboys, 500 l outdoor tanks, 5,000 l to 10,000 l outdoor tanks (Fig 3).



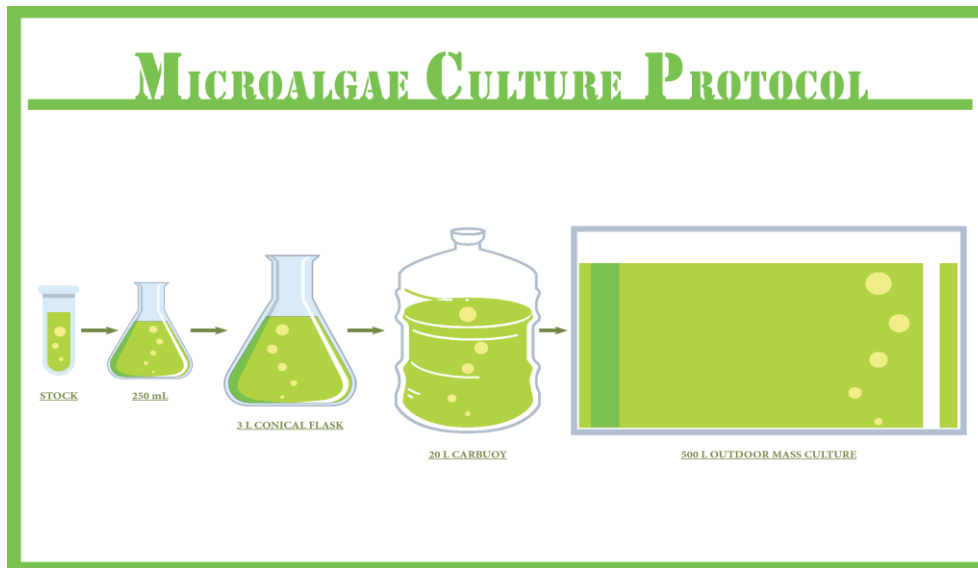


Fig3. Diagrammatic representation of batch culture of microalgae

- ✓ **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, *Chaetoceros calcitrans*, *C. gracilis*, *S. costatum* and *Thalassiosira pseudonana* whereas high concentrations of DHA are found in the *Pavlova lutheri*, *Isochrysis galbana* and *Chroomonas salina*. 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 21<sup>st</sup> century by the World Health Organization.

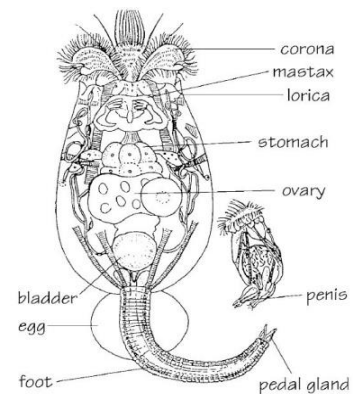
## Rotifer

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 microalgal 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.

### **Rotifer culture**

#### **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 @  $10 \times 10^6$  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 were obtained are the same. Place around 20 to 30 rotifers in to the test tube. Scale up the culture through 50 ml, 100 ml flasks 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**

The mass culture techniques discussed here are the ones generally used in the crab hatchery of CIBA, MES.

#### **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  $10 \times 10^6$  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<sup>th</sup> 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.

#### **Points to remember**

- 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**

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 penaeids, 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 zygotenic (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.

### **Procedure for hatching artemia cyst 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<sup>0</sup>C, 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.

### Procedure for Artemia production in salt pans (Outdoor culture)

1. Artemia production pond (100m<sup>2</sup>) 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<sup>0</sup>C.
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‰.
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‰ 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 O<sub>2</sub> level that prevails in high saline conditions. Non-selective filter feeder, tolerate wide range of salinity (5 ppt to 350 ppt) and temperature (6-35°C) 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

## Cysts Hatching Techniques

### Hydration

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

### Decapsulation

Decapsulation 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 vigorous 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  $29\pm 1^{\circ}\text{C}$  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.

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

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  $29\pm 1^{\circ}\text{C}$  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

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

### (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 hatchable nauplii

$T_{90}$  = incubation time all appearance of 90 % of total hatchable Nauplii

$T_s$  =  $T_{90} - T_{10}$ ; 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

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

### **Uses of Artemia in different forms for Aquaculture**

Artemia Decapsulated cysts : Directly using for Crab and Fish larvae

Just hatched nauplii : Shrimp, Fish and all cultivable species

II stage nauplii : Using for enrichment and feeding

Sub-adult : Feeding for post-larval stages

Adult : Nursery rearing, Used as ingredient for different types formulated feed for shrimp, fish and poultry

### **Copepod**

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 Platycopioidea, Calanoida, Misophrioida, Canuelloidea, Gelyelloidea, Harpacticoida, Mormonilloidea, Cyclopoida, Siphonostomatoida, Monstrilloidea. 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**

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, ricebran etc
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

## **Grow-out practices of Polychaete worm (*Marphysia gravelyi*) from the Muttukadu lagoon of East coast of India.**

Dr. Kannappan S., Navaneeth Krishnan, Dr. Jithendran K. P., Dr. Ezhil Praveena P. & Dr. Sivagnanam S.

### **Introduction**

Polychaetes (many hairs) are bristle-bearing multi-segmented worms of diverse class of Phylum Annelida. Polychaetes tend to form the dominant sediment dwelling fauna of most mudflats, estuaries and sheltered sandy shores. They also show an important role in ecosystem functioning (Baharudin *et al.*, 2014) as they have tolerance for low salinity and oxygen levels. Polychaetes constitutes as prominent zoobenthos which are diverse in nature and adaptable to various feeding habits, switching from carnivores to herbivores and detritivores to omnivores types. In the world approximately 8,500 Polychaete species were reported to be present (Glasby *et al.* 2000) belonging to 1,100 genera of which around 400 species were reported from India. Many species of polychaetes worms are able to adapt in aquatic sediments containing heavy metals associated with their food and they usually have capabilities to detoxify these metals in the gut and are less hazardous than absorption through the body wall (Berthet *et al.* 2003). They also show capability to control organic contaminants such as polycyclic aromatic hydrocarbons (PAH) and pesticides. Shrimp hatcheries have been extensively using polychaete worms as feed for shrimp broodstocks for facilitating fecundity. Polychaetes are described to have high content of polyunsaturated fatty acids, especially arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid (Bühning & Christiansen, 2001) that are required for the successful maturation and production of high-quality juveniles by both finfish and crustacean.

Commercial shrimp feeds, can intensify the waste load to the grow-out system, if not managed properly and also can deteriorate the water quality resulting organic pollution (Tucker and Hargreaves, 2008). The commercial feeds are regularly applied in the mono species grow-out systems, and their feed wastes are discharged without being used by other candidate aquaculture species. They may either be assimilated by physical, chemical or biological processes within the grow-out ponds or discharged as effluents (Boyd and Tucker, 1995). Therefore, mass collection of polychaete worms from natural resources and using them as feed for live fish or shrimp and also as baits would deplete them (Olive, 1993). Digging of soil for polychaetes collection and using them as baits is also a disturbance for feeding shorebirds coupled with the reduction in available prey items. Polychaete collection also causes physical disturbance and the return of heavy metals to the surface rendering them biologically available as well as the release of ammonia and phosphorus compounds from the sediments leading to eutrophication. The natural supply of polychaete worms has not been to be 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. Hence, the present study was carried out to develop grow-out practices for the macrobenthic polychaete worms for mariculture food.

## Preparation of sand bed and Collection of Polychaete worms:

Fiber-glass Reinforced Plastic (FRP) tanks (100 cm dia x 50 cm height) were selected and plastic couples of 4-5 numbers were provided from the bottom to the height of 10 cm. Aluminum substratum of 10 cm height was provided on the plastic couples. Nylon cloth culture bags (50  $\mu$ ) (80 cm dia x 50cm height) were placed on the substratum. Bottom aeration was provided through PVC square frame structure. Sea sand was collected and filtered in between 500 - 250  $\mu$  sieve. Then clay soil was collected from lagoon and filtered by 125 - 63  $\mu$  sieve. Sea sand and clay soil were mixed at 60 % to 40 % ratio and cleaned with potable fresh water and then by 10 ppt seawater and later sun dried for 3 days. Sandbed was placed in the culture bags in 10 cm height. Seawater was provided at 10 cm level and air-lifting water recirculation system was provided (Poltana et al 2007).



## Culture of *M. gravelyi*

Forty numbers of live adult polychaete worm's size ranging from 0.8 g to 2.0 g were stocked. Commercial shrimp feed (30%) of No: 1 was soaked overnight and passed through 50  $\mu$  filter net and sprayed for 2 times/day. Water exchange and was carried out in 15 days interval. Grow-out practice was continued for 120 days. Various water parameters such as salinity 25-30 PSU (practical salinity unit) ppt, pH 7.8 – 8.2 water temperature 21 -27°C were maintained.



## Culture of larvae of *M. gravelyi*.

Individual young *M. gravelyi* worms (5 Nos) (6-7 cm size of 2-3 weeks old) were collected from Muttukadu lagoon. The worms were stocked in separate plastic tubs (75-litre capacity) for 5 months with sea sand and clay soil bed (60:40 ratio) to 16cm level. Water level was maintained to 10 cm level from top of the sand bed with aeration. The worms were fed daily twice with 1% commercial shrimp feed.



## Mass culture of *M. gravelyi*.

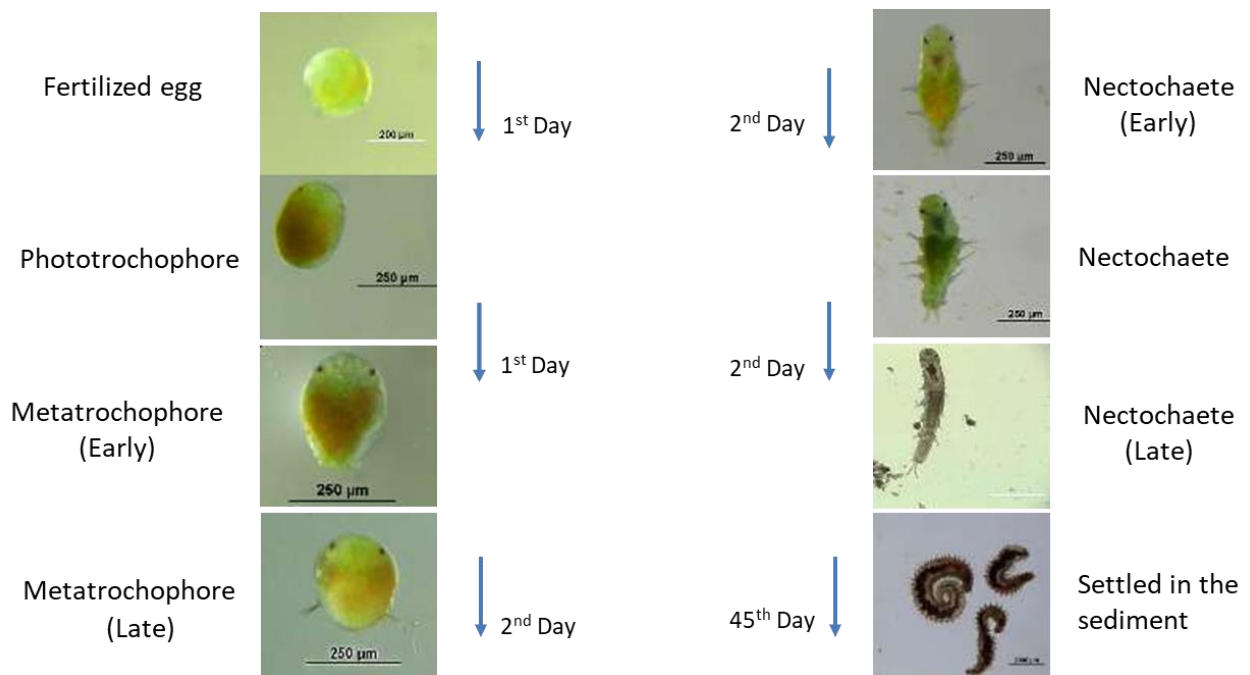
Four FRP tanks (2.0 m dia and 0.5 m height @ 1000 L capacity) were arranged then 15 nos of couplers are arranged at the bottom with 5 cm height. On the couplers, bamboo grill plate was placed. Above this nylon cloth bag (100  $\mu$  size) @ 2.0-meter level was tied. River sand 60 % passed through 75-micron mesh net and retained by 250 micron mesh net and lagoon clay soil 40% passed through 100-micron mesh net and retained by 63-micron mesh net. Both the soils were treated with 10 ppm Sodium



hypochlorite solution for 3 days. The soil was then washed in fresh water, later sun-dried and used as culture bed. Water filled 10 cm from bottom to the soil bed. Three sides airlifting flow-through system was arranged. Five hundred nos of *Polychaete* worms were stocked during August 2016. The worms were grown up for 3 months. Diatom *Skeletonema* & *Chaetoceros* was fed as feed @ 30 % body weight, 3ml per day. (Bharathidasan et al 2017)

### Developmental stages of *Marphysia gravelyi*.

#### Life cycle stages



#### Culture of adult Polychaete worms

When 40 numbers of adult polychaete worm's size ranging from 0.8 g to 2.0 g were cultured, a total of 2500 numbers of *M. gravelyi* was obtained (6 to 7 cm) from each tank with a 90 % survival obtained from each tank.

#### Culture of larvae of *M. gravelyi*.

Individual young *M. gravelyi* worms (5 Nos) (6-7 cm size of 2-3 weeks old) when cultured, the worms became adult (12 to 20 cm) in 5 months period. The worms made large burrows in the soil and produced egg mass and around 50-100 nos of 50 -60 mm larvae were found in two weeks of time from each worm after became adult.

#### Mass culture of larvae of *M. gravelyi*

Fifty numbers of young *Polychaete* larvae (6-7 cm size) of 500 nos were stocked in 1000-liter capacity FRP tank. Microalgae of *Skeletonema* spp at 1% body weight was fed with a water recirculation system. All the worms became adult in 5 months period (15 to 17 cm size). Approximately 3200 nos of biomass as adult worms were harvested from one tank with 80 % survival.

### Proximate Composition of *M. gravelyi*.

The protein, carbohydrate and lipid contents of *M. gravelyi* were estimated as 33, 14 and 40gm % respectively. The lipid content was reported as higher than others.

### Amino acid composition of *M. gravelyi*

SNo	Essential amino acids of <i>M. gravelyi</i>	g % (w/w)	Non-essential amino acids	% (w/w)
1	Leucine	11.90 ± 0.50	Cysteine	21.40 ±1.10
2	Lysine	10.90 ± 0.90	Proline	15.90 ± 0.90
3	Threonine	8.90 ± 1.00	Aspartic acid	8.80 ±1.10
4	Phenyl Alanine	2.80 ± 0.40	Glutamic acid	4.88±0.90
5	Histidine	2.10 ± 0.60	Asparagine	3.50 ±0.10
6	Valine	1.80 ± 0.40	Glycine	0.44 ±0.10
7	Iso- Leucine	1.98 ± 0.70	Serine	0.12 ±0.01
8	Arginine	0.002 ± 0.01	Alanine	0.04 ±0.001
9	Tryptophan	0.190 ± 0.02		
10	Methionine.	0.002 ± 0.01		
Values are average of three determination				

### Fatty acid composition of *M. gravelyi*.

Alpha-linolenic acid was reported as 30.48% (w/w), Linolenic acid: 27.42 %, Moroctic acid: 3.33%. Mono-unsaturated fatty acids were reported as 19.1% of oleic acid and saturated fatty acids were 14.0 % of Stearic acid; 3.98 % (w/w ) of Palmitic acid. Alpha-linolenic acid was reported as higher than other fatty acids.

### Conclusion

Polycheate, *Marphysa gravelyi* which was found large numbers in the Muttukadu lagoon area was identified as fast grown, higher rate of fecundity, easy acclimation and high lipid content. This species can be grown under mass culture in controlled condition and can be supplied to brood shrimp and fish as best live fish/shrimp food organism.

# **Grow out culture**

## **Prestocking, stocking and post stocking management in shrimp culture**

Dr. P. S. Shyne Anand, Biju I. F. & Dr. A. Panigrahi

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 grow out farming systems. There are various points to be taken care during the pond preparation for shrimp culture.

### **1.1. Drying the pond 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.

### **1.2. 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 is not generally recommended in acidic soils as it increases the soil pH.

### **1.3. Top soil removal:**

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



## 2. Liming

During pond preparation liming is applied to optimize pH and alkalinity conditions of soil and water. The type and amount of lime to be added depends mainly on the soil and water pH, which should be checked before lime application. The recommended levels of lime application during pond preparation are given in Table 1. The soil and water pH can be measured with a pH meter. Generally agricultural lime or dolomite can be applied if soils of pH >5 and Quick lime or hydrated lime can be applied if soil pH below 5. Where disinfectants like bleaching powder (calcium hypochlorite) is 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	13.8 to 11.5

## 3. Water intake

Stringent measures to be followed to prevent entry and growth of any unwanted and pathogenic agents in culture ponds. It can be achieved via proper filtration of intake water using appropriate mesh screens, disinfection of intake water. Generally bleaching powder @ 20-60 ppm is recommended for reducing the 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 production cycle.



**Table. 2. Optimum water quality criteria for intake water**

	Parameters	Range
1.	Temperature (°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

#### 4. Fertilization of pond water

The purpose of fertilization is to ensure the growth of primary producers in culture ponds. They initiate natural food web in the aquatic ecosystem and directly or indirectly contribute shrimp growth also. It also helps to maintain desirable level of transparency (25-40 cm) which prevents development of harmful benthic algae. Phytoplankton in culture ponds also help 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<sup>-1</sup> and inorganic fertilizers like urea and single superphosphate (SSP) at 25 – 100 kg ha<sup>-1</sup> 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. Brownish green colour of pond water indicate that the pond is ready for stocking the seed.

#### 4. Application of organic based indigenous probiotic

Yeast based organic preparation are 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 biweekly interval to improve the fertility status of water @ 1-2 ppm.

#### 5. Seed Selection and Stocking

Stocking of shrimp is one of the most important component 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 in well aerated tanks to remove weak PL. Maintain a balanced or optimum stocking density is also an important component of shrimp culture

## **6. Pond bottom and water quality management**

Disease outbreaks in shrimp growout culture are directly related to pond bottom and water quality. Zero water exchange must be followed throughout the culture period to prevent the disease occurrence. Use chemical like  $KmNo_4$  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

## **7. 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 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 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 reduce disease risks and during disease outbreaks. Proper storage of feed is also an important component in the biosecurity plan.

## **8. 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 colour. 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.

## **9. 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 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.

## **10. Better Practices for harvesting**

Harvesting must be avoided during moulting period and agricultural lime can be applied 3-4 days before harvesting. Try to do harvesting in the early morning or evening. Harvesting should be done with dragnet with minimum delay. After harvesting transport crates with crushed ice at 1:1 ratio for better preservation. Nutrient rich pond effluent must be treated before get discharged to water source.

# Penaeid shrimp nursery technology with special reference to *P. vannamei*: SOP and Scope

Dr. Akshaya Panigrahi

## **Introduction:**

The culturing system of brackish water shrimp comprises of hatchery, nursery and grow out system. Each stage plays an important role for the success in production. Nursery phase is defined as an intermediate step between hatchery-reared early postlarvae and grow-out phase. The main aim of the nursery phase is to produce large size juveniles, which will probably have a better chance of survival and may achieve commercial size in a shorter time (Apud et al., 1983). This phase gives several benefits in the shrimp production cycle such as, it increases the survival rate, improves the feeding efficiency, and it enhances the growth of shrimp. It has several advantages such as optimization of farm land, increase in survival, enhanced growth performance and reduction in the farm grow-out period. In order to achieve higher survival and reduce the grow-out period an intermediate nursery phase is essential. Nursery has been applied successfully in nursery phase in different shrimp species such as *L. vannamei*, *P. monodon*, and *F. setiferus*. There are many benefits of the use of a nursery during the grow-out phase, including more accurate stocking inventory, uniformity of shrimp size and reduction of cannibalism (Samocho et al., 2000; Yta et al., 2004). It has been established that bio-floc technology reduced occurrence of acute hepato pancreatic necrosis disease (AHPND, and IMNV in *P. vannamei* may be due to the antagonism which occurs between dense heterotrophic bacteria and *Vibrio parahaemolyticus* (Eksari et al., 2014)

## **Approaches:**

- In general, nursery systems may be one-phase, two-phase or multiphase operations and one phase nursery system is widely practiced.
- One and two phase operations may combine indoor and outdoor systems. Primary nursery system (indoor) is referred as the extended larval rearing phase.
- Separating 20 to 30% of the pond with a net as nursery and releasing shrimp into the whole pond after 20 to 30 days.
- Stocking in cages inside the ponds for the first 30 days with no soil contact which reportedly avoided an EMS outbreak is one approach.

However with these practices inside the pond compensatory growth does not happen since the shrimp are in the same pond.

The two biggest costs in shrimp farms are feed and duration of culture. The implementation of nursery systems has a significant direct impact in both parameters and also helps farmers to reduce risk and improve their profitability.

## **Success of nursery depends on following criteria:**

- Properly designed nursery with strict biosecurity
- Healthy recruitment (Post larvae) - WSSV, EHP and IHNV checking are mandatory

- **Stocking density** –optimum number (in primary nursery phase 2000 to 10000 nos/m<sup>2</sup> depending on the species, infrastructure and requirements).
- Biosecurity of the nursery system which can be operated with **Zero/minimal water exchange system**.
- Control mechanism of water conditions and shrimp health
- Optimization and better feeding efficiency.
- The compensatory growth capacity of the nursery reared animals

It may be taken up in autotrophic or heterotrophic way

### **Autotrophic versus heterotrophic microbial system**

Three predominant pathways for ammonia-nitrogen assimilation function in aquaculture systems. This includes photoautotrophic, chemo-autotrophic and heterotrophic system. The photoautotrophic system is mediated by algae and diatoms and mostly works at nitrate level, the last and the least toxic metabolite of nitrogen cycle. However, the other two systems (chemo-autotrophic and heterotrophic) system is mediated by bacteria and start functioning from ammonia level, the most important toxic metabolite in shrimp culture. The chemo-autotrophic microbial system is managed by aerobic *Nitrosomonas* and *Nitrobacter* and the end product is nitrate.

In contrast heterotrophic microbial system not only reduces ammonia level but also convert it into single cell microbial protein called biofloc. In addition to organic nitrogenous waste, ammonium will be converted into bacterial biomass if C:N ratio is balanced at a ratio of 10-15:1 (Schneider et al., 2005). The growth rate and microbial biomass yield per unit substrate of heterotrophs are higher than that of nitrifying bacteria, thus making many folds increase in heterotrophic bacteria (Hargreaves, 2006). Steve Surfing in 1976 put forward the 'microbial soup' concept that eventually led to development of "biofloc" based aqua farming. BFT was first developed in early 1970s at Ifremer-COP (French Research Institute for Exploitation of the Sea, Oceanic Center of Pacific) with different penaeid species and later Prof. Yoram from Israel, contributed immensely for the growth of this promising technology.

### **Advantages of Nursery rearing technology**

- Optimization of farm facilities provided by the high stocking densities in nursery phase to achieve more profitability
- It improves productivity and reduces costs without additional pressure in the production systems.
- Diurnal changes (pH, O<sub>2</sub>, CO<sub>2</sub> ) during nursery is reduced to give better performance
- Better nutrition by continuous consumption of high nutritious feed under autotrophic or in heterotrophic system positively influence grow-out performance as compensatory growth phenomenon proved.

- Post larval immunity stimulates under these systems thus giving healthy animals
- Reduced costs (15-20% lower cost of production) and better economic gain; Augmentation of natural food and improvement of FCR
- Heterotrophic bacteria can reduce toxic metabolites (NH<sub>3</sub>-N, NO<sub>2</sub>-N) in the nursery
- Easier management and environmental friendly approach (reduced protein requirement, fish meal usage and water/nutrient discharge)
- Increased protein utilization as the shrimp use proteins twice, Enhance digestion (with enzymes and growth promoters).
- More diverse aerobic gut flora reducing pathogenic bacteria (*Vibrios*) with probiotic intervention.

### **Biofloc based Nursery system:**

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<sup>3</sup> of water showing better performance in bio-floc system for *P. monodon* and *F. indicus* Nursery rearing under bio-floc system gives better result in terms of performance (growth and survival) and protective response.



BFT has been applied successfully in nursery phase in different shrimp species such as *P. vannamei*, *P. monodon*, *F. paulensis*, *F. brasiliensis* and *F. setiferus*. Zero exchange high density nursery reared juveniles found to have minimum size variation and better performance due to well-developed immune response systems. This is a very good biosecurity tool for the farmers and a perfect transition phase between the Hatchery and Farm.

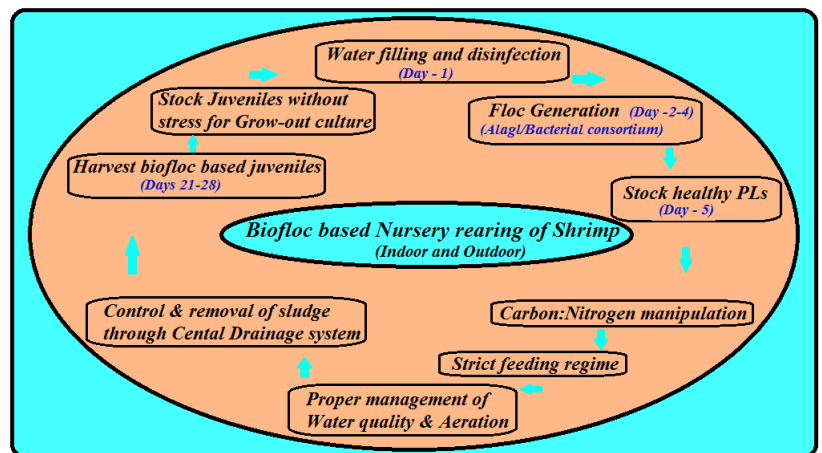
**Table 1. Growth performance of shrimps in Biofloc based nursery rearing system**

<b>Production performance</b>	<b>Control</b>	<b>BFT based Nursery rearing</b>
Stocking (Nos/m <sup>2</sup> )	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

### **Standard Operating Procedure:**

Biofloc based shrimp farming has a great opportunity to fine tune for better production, avoid disease occurrence with eco-friendly approach following standard operating protocols. SOP for nursery includes nursery design and construction, bio-security, suitable species, stocking (SD depending on system), culture management practices, feed management and carbon addition strategies, water quality management (zero/minimal water exchange), sludge removal etc..



### **Proper design and construction for nursery rearing**

Biofloc-based tanks/pond well prepared by lining (HDPE etc.) / concrete/ with designing of central drainage system can be used for effective utilisation and preventing erosion of dykes. Moreover, this helps sludge removal and serves as an effective control measure of the organic loading generated and prevent dyke breaching when the systems are aerated at a higher level. This technology can be conveniently applied in recirculatory aquaculture systems or raceway systems either with *in situ* inclusion or *ex situ* floc production through activated sludge system and putting the harvested bio-flocs in the production system. The system can be adequately agitated and aerated to keep the microbial floc in suspension.

***Raceways for shrimp nursery:*** Raceways are shallow (about 50 to 100 cm) and typically include a central baffle or partition to improve internal circulation. Water movement is provided by banks of air-lift pumps that draw water from the tank bottom and release it at the tank surface or by pumps that inject water through nozzles designed to provide aeration. Water is directed to flow along the tank in one direction and in the opposite direction on the other side of the partition. Raceways also have an extensive network of diffused aeration to maintain biofloc in suspension. At the highest intensities and standing crops, oxygen may be injected for a short time after feeding or continuously as needed.

***A central drainage system is highly beneficial for following BFT:*** Though biofloc system enables waste utilization, natural decay of floc and other materials becomes undesirable. These material can support the growth of pathogenic microorganisms particularly bacteria, fungi and parasites. Therefore, the pond should have a central drainage system to remove the settled materials from the bottom. Periodically these waste materials should be taken away through an efficient central drainage system.

### **Animal model and stocking density**

BFT technology is suitable for high density culture of species like *P. vannamei* which are effective at utilizing natural productivity and has been adapted with Tilapia production in aquaculture in the biofloc based culture system. Other penaeid shrimp species like *P. monodon*, *P. stylirostris* and *Macrobrachium sp.* are lesser suitable for the purpose, at high density. In nursery phase, higher stocking densities (5000 to 10000 PL/ ton) are used to reduce the production cost. This system will increase the number of crops in a year. However, the excessive increase in stocking density may decrease the growth and survival of the shrimp. This effect is associated with a combination of factors, including decreased viable space and availability of natural food, increased cannibalism, degradation of water quality, and the accumulation of organic matter in the bottom of the tank.

### **Disease monitoring programme**

It is very important to focus on the identification of all possible sources of pathogenic bacteria in nursery systems. The PCR based screening can be used to rule out any PLs with critical pathogen such as WSSV, IHNV and EHP etc. Animals should also be routinely observed for any abnormal behavior of animals. If any kind of disease is suspected, immediate care should be taken to prevent it or confine and avoid spreading of pathogens, if any.

### **Maintenance of stringent biosecurity measures**

Stringent biosecurity measures should be put in place to avoid any entry of pathogens from the external sources on horizontal route. The initial water used for culture practice should be filtered and treated sufficiently to ensure killing of all existing pathogens. The water then should get matured by addition of beneficial microflora. Ponds should be fenced properly. All the man, machine and material should be sufficiently sanitised while moving from one pond to the other during sampling.

### **Efficient feed management can avoid most of the problems**

The biofloc based system provides natural feed and therefore feed management like regular check tray observation and biomass driven accurate feeding is more desirable. 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). Any excess or unutilized feed can provide nutrients to many other pathogenic bacteria, fungi and parasites.

### **Indiscriminate use of chemicals/materials should be avoided**

As natural microbiota plays major role in BFT system, it is unnecessary to add any other materials, chemicals or antibiotics from the external source. The beneficial microorganisms of biofloc can act both as probiotics and bioremediators. The indiscriminate use of antibiotics/chemicals will kill the beneficial organisms along with the pathogens.

### **Research achievements and Case studies:**

- At ICAR-CIBA, in the pilot studies have achieved a number of milestones in conventional and BFT/BPT based nursery rearing technology including characterization of biofloc and periphyton, generation of biofloc, standardization 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 these system when compared to the conventional system (91-92%). For the biofloc generation in nursery system different inoculums like biofloc lyophilized powder or fermented biofloc product or recycled matured water can be used.
- In high density nursery trial of *F. indicus* and *P. monodon* (10000 nos / m<sup>3</sup>) is reported to yield better survival, 78% & 91 % respectively and growth (222 mg & 125 mg , respectively) (GAA, 2009).
- Priming of immune system of the host helps in immunomodulation and disease resistance in the animals reared in this system. Gene expression measured in mysis, postlarvae and adult *P. vannamei* was found to be enhanced in the presence of biofloc. Our studies suggest that microbes associated with bio-flocs may enhance expression of certain immune-related genes.
- *P. vannamei* PL grown in biofloc have shown less size variation and have been tested proved to have better immunity as per challenge tests conducted by ICAR-CIBA.
- The presence of bioflocs is reported to result in increase of 50% in weight and almost 80% in final biomass in *F. paulensis* early post larval stage when compared to conventional clear-water system.
- *F. brasiliensis* post larvae grow similarly with or without pelletized feed in biofloc conditions during 30-d of nursery phase, which was 40% more than conventional clear-water continuous exchange system.
- In recent years, more integrated groups in South and Central America have implemented a three-phase farming system with intermediary raceways/nurseries at pond site before stocking the animals into grow-out ponds, resulting in increased productivity by 20-30% and lowering production costs.
- Stocking post larvae (PL) after a nursery phase (usually >PL45) instead of PL10-12 direct from hatcheries will reduce the duration in the grow-out ponds by 20-30 days and feed conversion ratio (FCR) by 10-30%. In Mexico and Asia, this is now one of



the strategies used to mitigate early mortality syndrome (EMS) by stocking larger size post larvae into grow-out ponds.

- In Mexico, growth of 7-8 g in the first 30 days was achieved with the three-phase system as compared to 3-4 g with regular stocking showing that shorter cycle means savings in costs for feeds and energy.
- In Thailand and Malayasia adopting nursery gets mixed response. There was success at the nursery stage but due to the health condition of the animal during transfer, results were poor in the grow-out stage. So localized nursery or cage based nursery is also practiced.
- In Malaysia, proved that this change in strategy in which Post larvae were stocked in 500 to 1000-tonnes raceways at 0.6 to 1.8 PL/liter. Days of culture ranged from 30-57 days to produce 3.5 to 4.28 g juveniles which had very high growth rate survival with reduced survival in grow-out phase.

#### **Strategy for promotion:**

- Promoting the Nursery rearing phase to overcome the disease problem at early stages
- Working towards shift in policy for these environment friendly technologies with modifications in stocking density during nursery phase and restrictions on use of molasses as carbon source
- Development of bio-floc within various aquaculture practices –semi-intensive/super-intensive/RAS and adapting it for nursery phase
- Mechanism to control the unregulated growth of bio-floc in the existing pond systems and evaluating bio-floc and/or periphyton based waste for utilization as feed ingredient
- Extending the BFT/ BPT based nursery research outputs on other penaeid species for better understanding, since indigenous species show greater adaptation to local conditions and may be used in restocking programs or cultured in their natural environment
- Encouragement of this ecologically sustainable system through demonstration and capital subsidy and road map for the development should be prepared

#### **Conclusion**

Standard operation procedures must be followed for sustainable and eco-friendly nursery based shrimp culture systems. Not only do they enhance the production, cost effectiveness, but the prevalence of the disease occurrence is reduced in the aquatic environment. Major benefit accrues in the reduced feed cost for the farmers and increased profit through this technology. We need to work persistently on for the improvement of pond design and construction (central drainage system) developing system to indoor and outdoor trial and culture management practices, elucidate the composition of biofloc and their nutritive values, reduce the cost of production, Scrutinizing aeration methods in congruence with the oxygen

requirements in the BFT system and assessing the disease resistance, immunity of biofloc reared animals. Further, at policy level customizing the stocking density limits for nursery rearing and grow-out culture, the bio-security measures and making guidelines to encourage nursery based shrimp farming technology need to be brought out.

### **Suggested Readings:**

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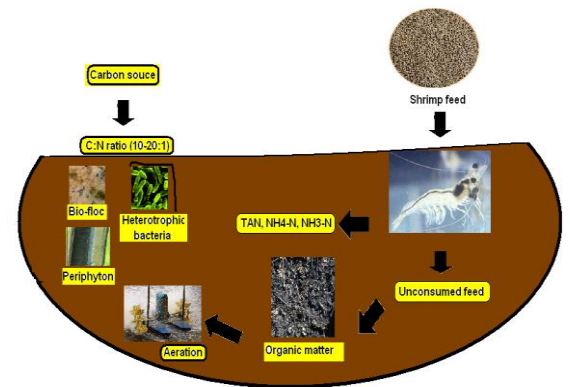
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# Eco-based culture management of Pacific white shrimp with special reference to Biofloc based farming

Dr. Akshaya panigrahi and Dr. Shyne Anand

## 1. Introduction

Eco-based technology like biofloc technology (BFT) in aquaculture is accepted more and more for improving the water quality, feed utilization, reducing stress and the disease susceptibility of the organism and cost of production, and lower the carbon footprints through efficient growth of heterotrophic microbes in the system. Biofloc is the conglomeration of microorganisms (such as heterotrophic bacteria, algae (dinoflagellates & diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans & detritus). By developing dense heterotrophic bacterial community, the system becomes bacterial dominated rather than algae dominated and takes care of the waste generated in the aquaculture system through *in situ* bioremediation. This innovative strategy for disease prevention and discouraging antibiotic, antifungal chemicals and other medicines application in aquaculture make it more acceptable as an eco-based farming for attaining higher productivity through sustainable approach. The Biofloc technology (BFT) technology had gained momentum and positive reviews in shrimp and tilapia farming.



The farmed shrimp production in India has grown from a production level below 1 lakh tonnes in 2009 to above 5 lakh tonnes in 2016-17 accounting for 38 % of the quantity and 64.5 % in value (Rs. 24,426 crores) of total sea food export worth \$ 5.78 billion dollar (Rs. 37,870 crores) export revenue (MPEDA, 2017). However, several major diseases are prevalent in India i.e. WSD, WFS, RMS, LSS, BGD, WMD and IHHNV and in Asian shrimp aquaculture diseases like Acute Hepatopancreatic necrosis disease (AHPND), Early Mortality Syndrome (EMS), *Enterocytozoon hepatopenaei* (EHP) and Running Mortality Syndrome (RMS), has reached epidemic proportion BFT approach promises a healthy rearing system, which is increasingly identified as one possible approach for disease prevention. Bio-floc system also allows the use of diets with lower crude protein (CP) content, which is supplied in part by the natural production associated with the formation of the bio-flocs. 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, thus reducing substantially the use of antibiotics.

## 2. 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*, *Litopenaeus vannamei*. Later, Prof. Yoram from Israel contributed immensely for the further modification

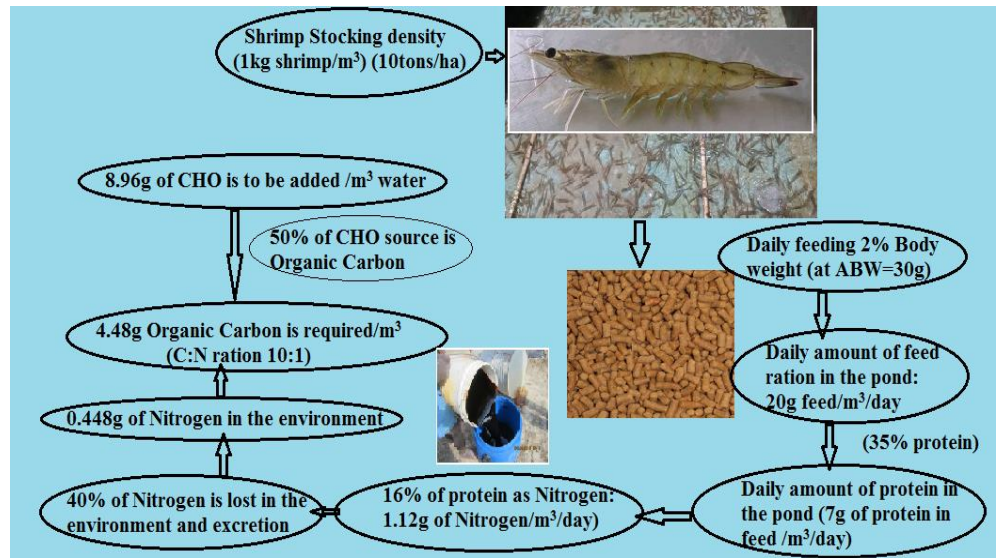
and promotion of this encouraging technology. The technique developed in Israel subsequently spread to many other countries due to its several advantages.

### 3. Biofloc constituents and Characteristics:

Bio-floc is the conglomeration of microorganisms such as heterotrophic bacteria, algae (dinoflagellates & diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans & detritus. By developing dense heterotrophic bacterial community, the system becomes bacterial dominated rather than algae and takes care of the waste generated in the system through *in situ* bioremediation.

#### **Microbial community:**

The characteristic feature of heterotrophic bacterial growth is its faster growth rate, as it almost doubles its number within 30 minutes. The bacterial efficiency of nutrient conversion is as high as 50%. At elevated C:N ratio, bacterial bio-floc recycles nitrogen to keep TAN below safe level



(1ppm) enabling shrimps/fish to be stress free in pond ecosystem. Based on the contributing microorganisms, three basic types, green, black and brown bio-floc systems have been categorized Brown flocs are more heterotrophic and gave better stability to pond, nutritionally better and more predictable. The number of total bacteria in bio-floc group was significantly higher ( $10^6$  to  $10^8$  cfu mL<sup>-1</sup>) than that of conventional system.

**Nutritional 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)

### 4. Attributes of Biofloc based farming technology

#### **Health advantages**

- Zero or minimal water exchange-** BFT encourages better biosecurity & pathogen exclusion.
- Stress-free environment-**Continuous aeration in the system mixes water thus avoiding stratifications and the stable water.

- c. **Diverse beneficial bacteria**-Bacterial community in the bio-floc can stimulate the non-specific immunity and limit establishment of Pathogenic strains
- d. **Probiotic action** - Bio-flocs can act as a natural probiotic which could act internally and/or externally against, *Vibrio* sp. and ectoparasites, diverse aerobic gut flora reducing pathogenic bacteria (*Vibriosis*).
- e. **Removal of settled and suspended solid waste**-These waste are removed from the bio-floc system thus preventing any risk of disease from the sludge.
- f. **Priming of immune system** of the host helps in immunomodulation and disease resistance in the animals reared in this system

#### Nutritional advantage

- g. **Natural productivity**-Augmentation of natural food and improvement of FCR
- h. **Protein is utilized twice**-Lower protein creates better heterotrophic environment
- i. **Nutrient rich**: 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
- j. **Broodstocks gonads formation**- Help in building reserve energy and superior reproductive performance.
- k. **Reduced costs (15-20% lower cost of production)** including 30-50% cost savings in feed

#### Environmental advantages

- l. Zero/minimal water exchange system.
- m. **Bioremediation**- Heterotrophic bacteria can reduce toxic metabolites (NH<sub>3</sub>, NO<sub>2</sub>)
- n. **Diurnal stress** -Diurnal changes (pH, O<sub>2</sub>, CO<sub>2</sub>) in water and in turn the stress is reduced
- o. **Environmental friendly approach**- reduced protein requirement, fish meal usage and water/nutrient discharge

#### 5. Grow-out culture system with BFT

Similar advantage in bio-floc based grow-out systems has also been reported by many studies. This is an excellent technology for getting a maximum production per cubic meter of water and square meter of land, depending on the sludge removal and aeration capacity of the system 2 to 6 kg of shrimp or 10 to 50 kg of fish can be grown in these farming systems. Continuous monitoring of DO and Floc levels is critical in these systems. 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, *L. 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. Ray et al. (2010) demonstrated that shrimp biomass production (kg m<sup>-3</sup>) was increased to 41% when

bio-floc concentration was managed. While hardly 1 to 2 , 3 kg shrimp/m<sup>2</sup> is harvested in conventional pond and BFT based system 4 to 5 kg/m<sup>2</sup> (Panigrahi et al.,2017), RAS based rearing systems, intensive shrimp production involving bioreactors and bio-floc technology have yielded >9kg shrimp /m<sup>2</sup> (Samocha et al., 2013). The bio-floc system also delivered more consistent survival rates, especially at higher densities.

**Floc 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 and then 6-20 ml/l later which is the nursery phase. 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 are 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<sub>2</sub> .

### **Biofloc generation in the culture system**

Biofloc generation is an important phenomenon for shrimp/fish culture in BFT system before or after stocking. There are different approaches to prepare the system for biofloc based shrimp farming depending on the design and compatibility of the system, the species to be cultured, intensity of farming and the buffering time for biofloc generation. Further, the system can operate with complete biofloc or semi biofloc mode with or without integrating substrates for periphyton growth.

Ponds are prepared following the Standard Operating Procedures (SOP) like drying, refilling with aged water/soil and disinfecting properly sieved through a 60-100 $\mu$  screen and also adhering to all biosecurity protocols. Autotrophic bloom is developed by fertilizing with nitrogen and phosphorous fertilizers and/or in combination with any other biofertilisers. The pH is maintained by liming with dolomite or hydrated lime. In the beginning, nitrogen level is built up using feed or any organic substance. Subsequently, CHO source like molasses or, rice bran, multigrain atta etc. is applied, depending on the feed quantity and the TAN level so that a high C: N ratio (15:1) can be maintained to stimulate the flocs. Typically, ponds start getting dominated by autotrophic algae and after a few days, the water turns brown and foamy as floc develops and the system becomes heterotrophic without much algae. Ammonia levels rises to a peak and thereafter falls, following the rise of nitrite which stabilizes after sometime and can be controlled by adding more C.

Once heterotrophic population establishes, the molasses or other carbohydrate additives can be regulated to keep high C: N ratio based on the TAN level and the feed quantity. There can be a modification in the process if the purpose is to generate biofloc in a shorter

duration. To hasten the process, biofloc inoculum from other ponds or its preserved form can also be applied. However, care should be taken before using any commercial inoculum for this purpose. Heavy aeration is required for keeping the floc under suspension. Pond liner allows easier maintenance of floc in suspension avoiding dead spots and stops accumulation of inorganic material from pond banks/walls caused by excessive water circulation.

## **6. Bioflocs constituents modulate immunity of host**

Biofloc technology can be seen more as a mechanism, which provides shrimps a chance to keep the immune system active at all times, as they get exposed to various microbes. Shrimps gut is exposed to natural micro flora, which provides nutritional, immunological benefits, especially on preventing the infection from the pathogen by competitive exclusion, neutralization of toxins and bactericidal activity. The natural probiotic effect in biofloc provides antigen to trigger the immune response in gut. Many reports suggest that wide range of beneficial microbes or their cell wall components and metabolites is improving the innate immunity and can be employed in the health management of fish and shell fishes (Panigrahi, et al. 2007, 2017 and 2018). Biofloc is been proved that it is rich in natural source for bioactive compound and microbes thus it is proved to be an efficient immune inducer in the shrimp. Biofloc increases the survival rate even when there in an infection.

Constituents of bacterial cell walls in biofloc (components activate a cascade of reactions leading to the production of prophenoloxidase system and other biochemical pathways. The immune parameters like phenoloxidase activity, total hemocyte count (THC) are found to be increased in response to bio-floc treatments (Panigrahi et al, 2017). The upregulation of immune genes in our study indicates that the bacteria associated biofloc play the major role in enhanced immune activity in the shrimps. Gene expression measured in mysis, postlarvae and adult *L. vannamei* was found to be enhanced in the presence of bio-floc.

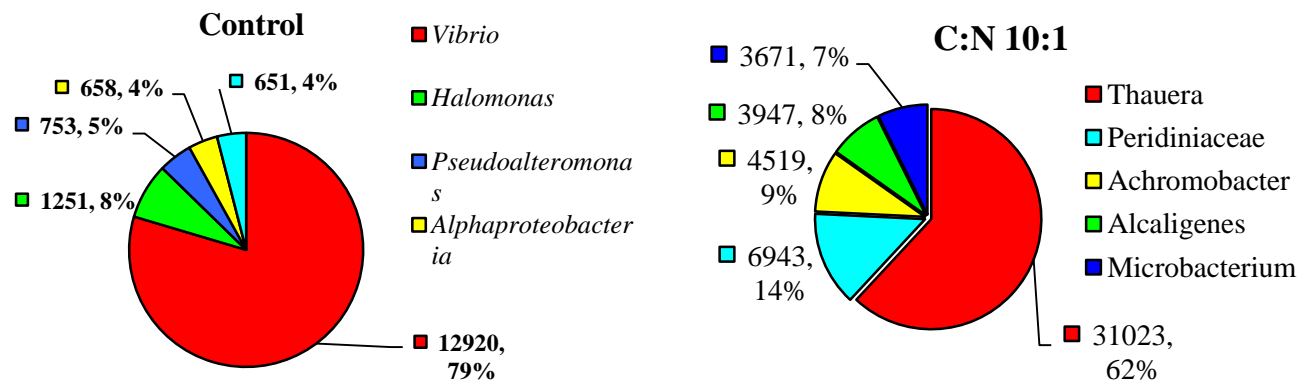
Our studies suggest that microbes associated with bio-flocs may enhance expression of certain immune-related genes. Transcripts of target immune genes were measured by qPCR. Which revealed appreciably enhanced mRNA expression of immune genes like SOD, Propheoloxidase, Hemocyanin, lysozyme, serine proteinase and glutathione peroxidase upregulated at different fold expression in *vannamei* reared in biofloc nursery system. Superoxide dismutase, prophenoloxidase in *L. vannamei* reared in biofloc systems. Studies in the similar line also revealed an up-regulation of immune genes with exposure to bio-floc thus implying immunomodulation in the shrimp (Panigrahi et al. in press). Gene expression analysis showed that the transcripts of those immune genes were significantly increased among all C:N treatments than that of control

### ***Biofloc as biocontrol agent:***

The “natural probiotic” effect in bio-floc could act internally and/or externally against, i.e., to *Vibrio* sp. and other disease agents. Antagonistic properties of bio-flocs can be explored to understand its mechanism to control the infection. The regular addition of carbon in the water is known to select for polyhydroxyalkanoates (PHA) accumulating bacteria which produce biodegradable polymer storage products, like poly-β-hydroxybutyrate (PHB), having antibacterial or biocontrol properties that provide immunity to the host. Beneficial communities in biofloc system control the pathogenic *Vibrio* population (Crab et al., 2012).

The PHB particles offer preventive and curative protection in *Artemia* nauplii against luminescent pathogenic *Vibrio campbelli*. Biofloc microbial community may involve in Quorum sensing, bacterial cell-to-cell communication with small signal molecules and is proposed as new strategy to control bacterial infection in aquaculture.

**Metagenomic approach for biofloc characterisation:** In one of our experiment to understand the bacterial constituent of biofloc we have demonstrated the relative abundance of the most frequently identified bacterial phyla in water from different rearing conditions (Panigrahi et al. 2018) as depicted in the following figure (Fig. 3)



(Fig 3: Clear water and biofloc (BFT) with CN ratio. 1A - Control; 1B - C:N 10)

The diversity of bacterial flora was more spread as the C:N ratio goes up in the treatments with as the major groups. The trend of *Vibrio* dominance decreased with the increase in C:N ratios and thus confirming the dominance of heterotrophic bacteria in high C:N ratio groups. *Vibrios* often considered as opportunistic pathogens, where the most dominant bacterial flora of water in control (79%) and C:N 5 (37%) group. In C:N 10, *Thauera* (62%) was most represented genus.

### **BFT and protective response**

The cell wall components of these beneficial microbes have potential immunomodulatory properties. Recently it has been established that bio-floc technology reduced occurrence of acute hepatopancreatic necrosis disease (AHPND, also called early mortality syndrome, EMS and IMNV in *L. vannamei*. This may be related to antagonism between antagonism occurs between dense heterotrophic bacteria and pathogenic *Vibrio*, the causative agent of AHPND

### **7. Bio-flocs for in situ bioremediation:**

The biofloc system maintains adequate water quality especially toxic nitrogen metabolites. At higher C: N ratio, bacteria immobilize toxic ammonia into microbial protein within few hours as compared to slow conventional nitrification process which takes a month to get established. By having bio-flocs in culture water, the utilized nutrients could be continuously recycled and reused. Then this environmental friendly "Bio-floc Technology (BFT)" could be a closed recirculating aquaculture system or a zero water exchange system, and is considered as an efficient alternative system for conventional pond based farming.



### ***Probiotic driven biofloc system***

Antibiotics and other chemotherapeutic 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 day, 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 and biofloc can be customized with proven probiotics to make it more efficient. 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.

### **8. Microbial biomass application as feed**

The macro aggregates of bio-floc are protein-lipid rich natural source available “in situ” throughout the day (Avnimelech, 2007). It is possible to replace one third of a conventional diet by low-protein bio-floc meal without interfering survival and performance of the shrimp. Harvested bio-flocs have been biochemically characterized as premium quality feed ingredients. The nutritional quality of bio-floc to cultured animals is good but rather variable. The dry-weight protein content of bio-floc ranges from 20 to 50 percent, with most estimates between 25 and 45 percent. Fat content ranges from 0.5 to 15 percent, with most estimates between 1 and 5 percent. There are conflicting reports about the adequacy of bio-flocs to provide the often limiting amino acids methionine and lysine. Bio-flocs are good sources of vitamins and minerals, especially phosphorus

***Microbiome analysis:*** Microbiome study reveals that with heavy rain as well as in disease situations like AHPND/WSD, bacterial richness of the gut declines substantially, which may be putting the shrimp under stress and suppress their immunity. Recently, lot of interest has been generated to study and understand the gut microbiome. In severe disease conditions, dysbiosis or an imbalance in the gut bacterial community occurs. The nutrient availability to the stomach could be altered so as to improve the metabolic function of the microbes in any stressful conditions. In this situation a customised microbial richness help to pass through the situation.

### **9. Research activities and achievements**

- At CIBA-ICAR achieved a number of milestones in biofloc and periphyton based nursery and grow-out farming technology through a number of pilot scale and collaborative studies. 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 BFT system compared to 2.5 to 3 kg/ cu m in conventional autotrophic system (Panigrahi et. al, 2014, 2017).
- 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. *P. vannamei* PL grown in Biofloc have shown less size variation and have

been tested proved to have better immunity as per challenge tests conducted by ICAR-CIBA.

- In one of our CN ratio standardization experiment, CN ratio of 15 was found to be the optimum one for better performance of *L. vannamei*. Metagenomics study revealed that *Vibrio* dominance decreased with the increase in this ratios (Panigrahi et al., 2018) and thus confirming the dominance of heterotrophic bacteria in high C:N ratio groups.
- We are 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.

## 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 technology has potential to revolutionize the aquaculture sector, this is still in an initial stage and lots of research is necessary for its modification, standardization and implementation. Bio-floc technology with biosecure modular systems may be an answer for more efficient, sustainable, profitable aquaculture production.

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# **Recirculating Aquaculture systems for *Penaeus vannamei* farming**

Dr. P. Nila Rekha, Biju I. F. & Jose Antony

## **Introduction**

Intensification of farming practices together with the steady increase in the demand for fish has pushed the aquaculture industry to look for acceptable practices from environmental, societal and economic perspectives. Recirculating Aquaculture System seems to be the solution as it could meet the requirements of environmental protection and resource management simultaneously.

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. The concept of RAS is to reuse a volume of water through continual treatment and delivery to organisms being cultured. 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.

The RAS has the following advantages: a) lower water requirements, b) lower land requirements, c) reduced labor requirements, d) increased control over water quality parameters, e) lower risk of negative impact from adverse weather conditions, f) lower risk of creating adverse environmental impacts, and g) increased biosecurity. These systems are being deployed in developed countries where coastal land costs and labor costs are very high. RAS systems have become very popular for fish culture especially for intensive cultures. Shrimp farming, which is plagued with disease outbreaks, also look forward for introducing RAS systems both in the maturation systems in hatcheries and in land based tank and pond culture systems

## **Components of RAS**

Recirculating aquaculture system consists of some typical components which have certain treatment process.

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

### **1. 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 impact on water quality.

- Settleable solids: Probably the easiest to remove, and should be removed from the system as quickly and frequently as possible. It can be removed as they accumulate on the tank bottom through properly placed bottom drains or keep them in suspension with continuous agitation and removed with sedimentation tank or mechanical screen filter.
- Suspended solids-. The most popular treatment method for removing suspended solids involves mechanical filtration with screen or granular media (sand or pelleted media). The effluent water with solids passes through a fine-mesh stainless steel or polyester screen, particles impinge on the screen, and as it becomes clogged, a high pressure backwash flow removes the particles from the flow-stream and exits as wastewater. The most common screen filter is the drum filter because it can be adjusted to solids loading, it has a larger surface area than standard disk filters, and it not likely to collapse under high loading rates of solids. Granular media filtration removes suspended solids by passing wastewater through a bed of granular media, such as sand or plastic beads, where the solids either adhere to the media or become trapped in the interstices between media. As the media becomes clogged, solids are removed by a “backwashing” process at which the media is subjected to high-pressure, reverse flow causing the bed to expand and “boil”, thus releasing the solids through a waste flow-stream.
- Floatable solids: It can be removed easily by fine meshed scoop net. Fine suspended solids (< 30  $\mu\text{m}$ ) contribute more than 50% of the total suspended solids increase  $\text{O}_2$  demand of the system and cause gill irritation and damage in finfish. Dissolved organic solids (protein) can contribute significantly to the  $\text{O}_2$  demand of the total system.
- Fine and dissolved solids: It can be removed by foam fractionation (also known as protein skimming). Simply, air is bubbled up through a closed column resulting in foam at the surface. Dissolved organic compounds (DOC) are physically absorbed by the bubbles, and fine solid particles become trapped in the surface foam and can be removed.

## 2. Biofiltration for nitrification

Ammonia and nitrite-nitrogen are byproducts of the metabolism of protein metabolism in feeds (fecal material and decomposition of uneaten feed). If un-ionized ammonia ( $\text{NH}_3$ ), and to a lesser extent nitrite, are allowed to concentrate in the culture system, they will become toxic to the animals in 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.

## 2.1 Rotating Biological Contactor

Rotating biological contactor (RBCs) have been used in municipal wastewater treatment systems for decades, but recently was adapted for aquaculture systems. RBC technology is based on the rotation of filter media attached to a shaft partially submerged in water (@ 40% submerged) (Figure 1). The nitrifying bacteria coat the surfaces of the filter media, and as the cylinder rotates, it will spend 40% of the time submerged and 60% exposed to air. This is an important aspect of all biological filtration. The oxidation of ammonia/nitrite-nitrogen requires a great deal of oxygen (known as BOD or “Biological Oxygen Demand”), and without abundant availability of oxygen the biofiltration is compromised.

Therefore, the alternate submergence in nutrient rich water, followed by exposure to the atmosphere, makes the RBC a very efficient biofiltration system. RBCs can be rotated by a motorized, gear-driven engine attached to a shaft, or they can be designed to be turned by water, similar to a water wheel, provided using an air-lift pump. The advantages of RBCs are

simplicity of operation, oxygenation and degassing CO<sub>2</sub>, and self-cleaning capacity. Disadvantages include high capital costs and a tendency for mechanical problems (associated with the increased weight of the contactor, which increase 10-fold over time).may

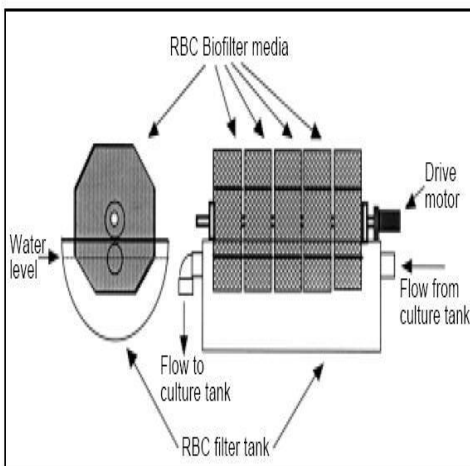


Fig. 1 A rotating biological contactor (RBC) unit powered by an electrical motor

the media. Since the filter media in trickling systems are not submerged, they not only provide biological filtration (nitrification) but also aeration and removal (or degassing) of excess carbon dioxide (CO<sub>2</sub>).

## 2.2 Trickling Filters

Trickling filters are also an offspring of municipal wastewater treatment systems. This type of filter is comprised of media with a low specific surface area allowing for large voids (air spaces) within the media. Wastewater is delivered at the top of the filter, usually with a rotating distribution bar, and gravity feeds through

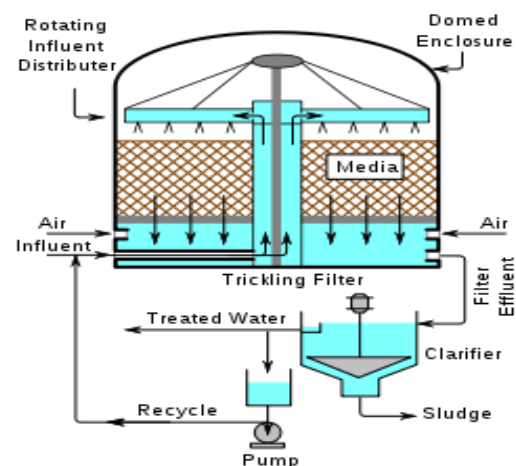


Fig 2. Trickling filter system

### 2.3. Fluidized Bed Filters

These are essential mechanical sand filters operated continuously in the expanded (backwashing) mode so that the sand media becomes fluidized. An up flow of pressurized water to keep the sand grains in motion, and not in continuous contact with one another, providing an excellent substrate for nitrifying bacteria that allows the entire surface for colonization. In most cases, fluidized beds use a fine-grained sand (finer than typical mechanical sand filters), and in some cases plastic beads have been used. Usually, fluidized sand filters are tall columns, which minimize their footprint in the facility. Other advantages include the low cost of sand as a filter media, compared to plastic beads, rings, etc., and its high efficiency of removing TAN should be maintained at < 20 ppm to maintain good growing condition.

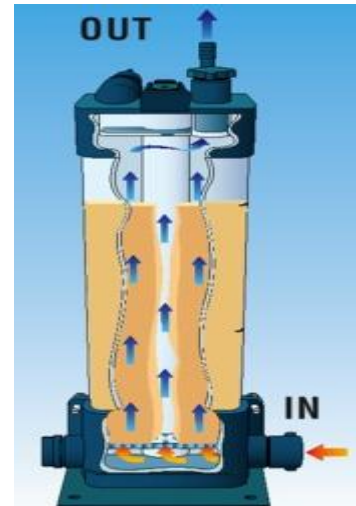


Fig 3 Fluidized bed filter

### 3. 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 blower will be necessary to perform this task. Excess CO<sub>2</sub> can be toxic to aquatic species and therefore bio filtered water must go through a degasification chamber such as a packed column to prevent accumulation. CO<sub>2</sub> is a byproduct of fish/shrimp and bacterial respiration and it can accumulate within recirculating system should be maintained at < 20 ppm to maintain good growing condition. Elevated CO<sub>2</sub> concentrations in water are not toxic when sufficient DO is present.

### 4. 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. 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.

**4.1 Ozonation:** Ozone (O<sub>3</sub>) gas is a strong oxidizing agent that has been used to treat municipal water supplies for years. In aquaculture systems with high levels of dissolved and suspended organic materials, the efficacy of ozonation may be limited. The efficiency of ozone to disinfect is dependent upon contact time with the microorganisms and the residual concentration of ozone in the water (after oxidizing all of those dissolved and suspended organics). Ozone has been used in RAS to control pathogens to oxidize NO<sub>2</sub> to NO<sub>3</sub>, organic matter, TAN or fine suspended particles. Ozonation improves micro screen filter performance and minimizes the accumulation of dissolved matter affecting the water color.

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.

**4.2 U.V. Irradiation:** Ultraviolet (UV) radiation can be used to destroy ozone residuals and to 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 as a florescent light bulb) is housed in a quartz cylinder, which is 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 advantages 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 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 needs 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 they increase production of nauplii and improve 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.

### **RAS for shrimp grow-out culture**

The shrimp species that is best-suited to high intensity inland aquaculture systems is the *Penaeus vannamei*. Pacific white shrimp perform well at high densities and at low salinities (Van Wyk et al., 1999; Samocha et al, 2004). Recent studies and observations suggest that strategies for culturing shrimp in recirculating system should include a mechanism for developing and maintaining suspended organic material within the culture



tank. This represents a fundamental departure from the traditional recirculating aquaculture water treatment paradigm.

Ammonia is the primary excretion product of protein metabolism and is excreted by shrimp as unionized ammonia. Unionized ammonia is highly toxic to the shrimp and must be removed from the system. There are potentially three different pathways for ammonia removal in a zero-exchange system:

- nitrification by autotrophic bacteria;
- assimilation by heterotrophic bacteria; and,
- Assimilation by photosynthetic algae.

In any given aquaculture system the types of bacteria and/or algae that develop and contribute to ammonia removal will be a function of the management regime. The principle variables that determine the bacterial or algal composition of this system include: solids removal rate, quantity of surface area provided for nitrifying bacteria, C: N ratios of the feed, alkalinity of the water, oxygen and light intensity. Accordingly the management regime in RAS system may be

**Chemoautotrophic Production Systems:** Recirculating aquaculture systems for finfish are typically designed and managed to promote the dominance of chemoautotrophic nitrifying bacteria. Because of the poor growth performance of shrimp raised in these systems, pure chemoautotrophic systems do not appear to be a good choice for shrimp production.

**Heterotrophic Production Systems:** Pure heterotrophic production systems are characterized by little or no water exchange, limited removal of solid wastes, in a pure heterotrophic production system there is any need for a fixed-film bio filter because the ammonia-nitrogen is controlled at very low concentrations by heterotrophic bacteria. In heterotrophic shrimp production systems the key management tools will be the manipulation of sludge wasting rates to maintain optimal TSS levels in the culture tank.

**Photoautotrophic Production Systems:** In systems dominated by algae, ammonia-nitrogen and nitrate-nitrogen are controlled by direct uptake and assimilation into algal biomass. Solids filtration is another strategy that has been incorporated into photoautotrophic shrimp production systems in an attempt to minimize the frequency and severity of phytoplankton crashes

**Hybrid Production System:** In reality, no system is purely chemoautotrophic, heterotrophic, or photoautotrophic. However, it is possible to manage a system to intentionally balance the presence of chemoautotrophs, heterotrophs, and photoautotrophs, all at the same time, or in various combinations. The design of a chemoautotrophic/heterotrophic (C/H) production system will resemble that of a traditional chemoautotrophic system. The main elements of the filtration system include some type of solids filter and a fixed-film biofilter. By operating these systems at lower C: N ratios than are necessary for full heterotrophic control of ammonia-nitrogen. Special attention must be paid to the design of the biofilter in C/H production systems due to the high TSS levels. Biofilters for these types of systems should be resistant to fouling.

## Water circulation in RAS and Pump selection

Water circulation In RAS, water is usually circulated by pumps to move water to a higher elevation or to increase the overall system pressure for filtration, aeration and degassing. Depending on a system's hydraulics, there are two RAS types: pressurized or high-head systems and low-head systems. The advantage of a pressurized or high-head RAS is the hydraulic link between source and the point of discharge, which is relatively independent of the pipe's geometry. However, a change in flow at one distribution point will influence flow at another point. In such system centrifugal pumps are used. The efficiency of such pumps depends on the impeller's design (e.g. open, enclosed, single or double suction, vane configuration) which can limit the size of solids that pass through the pump. In contrast, low-head RAS present the advantage of moving large volumes of water using significantly lower energy, improving the economic returns of investment .In such systems, either airlift pumps, axial-flow propeller pumps or some combination of the two is used. Due to limited head capacity, airlift pumps have has been generally thought to be insufficient to provide the water treatment requirements of high-density large capacity RAS, while axial flow pumps may be efficient at moving large volumes of water to modest head levels (e.g. 4.6–9.2 m) and tolerable to small debris and solids. The main disadvantage with airlift pumps is the low water delivery height, which is limited to a maximum of around 0.3 m. In those cases, the energy needed could be reduced by 40% compared to centrifugal pumps. The head loss in most RAS is a limiting factor; operating costs can increase 20–40% at 1 m pumping head and over 44–69% at 3 m head comparing to traditional flow through systems.

Due to the high operational costs of pumping airlifts are becoming more common; they are simple to use and economic under a limited set of operating conditions. Moreover, this equipment can serve for water transport, gas exchange and foam fractionation, which may have some advantages when compared to other pumping methods, such as a lower occurrence of breakdowns, a reduction of the need for technical supervision, and a reduced use of space. Energy costs of airlift pumps when used for low head water transport and aeration have been up to 35% lower when compared to standard pump and standard aeration combination

Pump selection must be done to match the RAS hydraulic profile. There are various types of pumps available.

- centrifugal,
- axial flow,
- Air lift.

In general, axial flow pumps can be more hydraulically and energy efficient. Properly selected and trimmed, low-head centrifugal pumps are needed for higher head systems. Furthermore, variable frequency control is an alternative to trimming impeller. Nevertheless, in a real production, pump selection is highly dependent on flow rate and/ or head requirements. Additionally, the availability of pumps to match required flows may be limited. Thus, it is difficult to recommend a single type of pump. A suggested solution, resulted from this study, may be an axial flow pump with variable frequency control.

**Pond based recirculation aquaculture system:** The RAS system could be pond based also with lined ponds and suitable filtration systems. The various components of pond based recirculation system are good influent water, appropriate pumps, reservoir tanks and filtration system.

## Conclusion

Research and development in recirculating systems has been going on for nearly three decades. There are many alternative technologies for each process and operation. The selection of a particular technology depends upon the species being reared, production site infrastructure, production management expertise, and other factors. Prospective users of recirculating aquaculture production systems need to know about the required water treatment processes, the components available for each process, and the technology behind each component. The dietary importance of microbes and detritus for *P. vannamei* is driving the development of new management regimes for culturing this species in recirculating aquaculture systems. The lessons learned from zero-exchange intensive shrimp ponds are being applied in ultra-intensive indoor recirculating aquaculture systems. The idea of operating a recirculating production system with the intent of promoting, rather than limiting, the development of heterotrophic bacterial populations, and managing ammonia without being dependent upon nitrifying bacteria, represents a paradigm shift for recirculating aquaculture. There is still much to be learned about how to manage and control these systems to increase system stability and profitability. Studies will need to be undertaken to determine optimal TSS levels, optimal solids wasting rates, how to control the composition of the microbial flocs, and how best to control the accumulation of carbon dioxide and stabilize pH

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## **Shrimp farming using inland saline groundwater with special reference to saline lands in North India**

Jose Antony, Dr. P. K. Patil, I. F. Biju, R. Aravind, Dr. A. Panigrahi and Dr. C. P. Balasubramanian

### **Background**

Vast resources of underlying saline groundwater of inland origin in the north western states of India form an attractive proposition for shrimp farming after amendments in the ionic profile of the growing media. Inland saline groundwater is ubiquitously deficient in potassium and has an excess of calcium and magnesium compared to seawater diluted to the same salinity. However, a disproportionately higher calcium level results in a lower  $Mg^{2+}:Ca^{2+}$  ratio (1.5:1 to 2.1:1) and the potassium deficiency creates very high  $Na^+:K^+$  ratio (250:1 to 300:1) making saline groundwater far different from that of seawater. Shrimps cannot grow or survive in raw saline groundwater wherein the  $Na^+:K^+$  ratio exceeds 200:1, and therefore commercial operations make use of potassium supplementation using agriculture grade muriate of potash (KCl).

Tiger shrimp was the main stay of Indian brackishwater aquaculture ever since the industry emerged in the late eighties and early nineties. The species is often termed to as the giant tiger shrimp owing to its fast growth rate and large size attained. The species has a wide salinity tolerance and commercial operations are feasible even between 1 to 5 ppt. Tiger shrimp has an average weekly weight gain of 3g/week. Tiger shrimp farming in India nearly collapsed in 1994-95 due to the outbreak of white spot disease owing to the unscientific farming practices followed. The post white spot period witnessed better adoption of BMPs and scientific farming practices resulting in peak productions during 2004-05. The latter half of the first decade of the new millennium witnessed a serious drop in tiger shrimp production in the country due to lower growth rates (1 to 1.5 g/week or lower), recurring incidences of white spot, lowered survival, stunting and poor productivity of the farming.

Similar trends were already visible in tiger shrimp farms of China and other south East Asian countries forcing them to import the Pacific white shrimp, *Penaeus vannamei* prior to 2000. SPF broodstock of white shrimp was first imported to mainland china in 1998 and this reversed the scenario of the shrimp farming industry from the domination of black tiger shrimp to the pacific white shrimp. Learning from the successes and risks of the new species which showcased promising results, the GOI permitted the import of SPF vannamei in 2008 and farming commenced in 2009 under strict guidelines of the Coastal Aquaculture Authority (CAA). Greater productivity, the availability of disease free seed, better growth rates, higher stocking densities and higher productions made vannamei the species of choice for coastal famers. The sudden boom in *P. vannamei* farming side-lined tiger shrimp, resulting in a handful of hatcheries and a few hundred farms rearing tiger shrimp. However, off late the industry is facing a crisis scenario in *P. vannamei* farming due to poor growth rates mostly due to genetic inbreeding as a result of use of pond reared broodstock and greater incidences of white spot outbreaks. As a result there are signs of the industry coming back to tiger shrimp and other indigenous species. The availability of SPF broodstock of tiger shrimp can pave way to disease free seed which when coupled with sustainable farming practices and biosecurity revive the industry. The inland saline areas of Haryana wherein multiple successful crops of tiger shrimp was taken and demonstrated to the stake holders,

form ideal sites for revival of the indigenous tiger shrimp farming in the country along with the states of Punjab and Rajasthan.

## History

Black tiger shrimp being the mainstay of Indian brackishwater aquaculture scenario until 2009, also formed the species of choice for preliminary inland saline trials in Haryana. Farm trials were directly conducted for *Penaeus monodon* in unamended raw saline groundwater at Sultanpur in Haryana and promising results were obtained (Dwivedi and Lingaraju, 1986). Interestingly, the saline groundwater at Sultanpur had a higher concentration of potassium making potassium supplementation unnecessary. However, most sub surface saline groundwater of inland origin have poor potassium ion levels and a high Na<sup>+</sup>: K<sup>+</sup> ratio resulting in complete mortality of tiger shrimp PL in 24 to 72 hours. Feasibility trials on tiger shrimp farming continued at the Central Institute of Fisheries Education (CIFE) centres in Rohtak, Haryana and Udaipur, Rajasthan.

The first successful commercial farming demonstration of tiger shrimp was carried out at the Rohtak centre of CIFE in 2008 with an average production of 660 Kg/ha (ICAR, 2009; Purushothaman et al., 2014) following which a better production of 1340 Kg/ha was reported in 2009. Following the success of tiger shrimp farming at Rohtak successive farm trials and indoor experiments were conducted to refine the technology and to upscale it (Raizada et al., 2015; Antony et al., 2015). Based on observations from the indoor trials, the first low saline crop of tiger shrimp was taken in 2012 wherein the salinity was maintained lower than 5 ppt throughout the culture period and large sized prawns were harvested (Lakra et al., 2013). The first demonstration of tiger shrimp farming in farmer's pond was carried out in 2013 under NABARD-SDC rural innovation fund. Interestingly, the first crop of Pacific white shrimp (*P. vannamei*), was also taken in the year of 2012 following which farmers have shown greater interest in the species owing to its higher stocking density, greater production and returns.

Following the success of *P. vannamei* in Rohtak, as on today tiger shrimp farming is absent in the state with not a single stake holder opting for the species. Commercial inland saline shrimp farming in the country is presently dependent on a sole species i.e. *P. vannamei*. Shrimp farming in inland saline water is same as that of coastal areas except for certain inputs which are essential for inland saline areas. The following section discusses the additional inputs required for farming shrimp in inland saline water and its implications.

## Ionic make up of inland saline water

The ionic profile of inland saline groundwater is dependent on the salt bearing rocks present underneath the soil which in turn creates saline groundwater. The ionic makeup of these rocks are variable and thus saline groundwater around the world vary in its ionic makeup to the extent that water obtained from wells in the same region possess a variable ionic makeup. The major cations present in saline groundwater is Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and the anions are Cl<sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, HCO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup>. Saline groundwater around the world is lacking in K<sup>+</sup>, Mg<sup>2+</sup> and SO<sub>4</sub><sup>2-</sup> compared to seawater diluted to the same salinity. Saline groundwater in north western states of India is also lacking in these ions and usually has an excess of Ca<sup>2+</sup>. The levels of Na<sup>+</sup> and Cl<sup>-</sup> are almost similar to seawater levels in most saline groundwater, though there are a few sites in which the chloride levels are high making

it unsuitable for shrimp farming. The ionic make up of inland saline water from different locations is depicted in the table.

When farming shrimp in low salinity inland saline water the ionic composition of the water is more critical than the salinity. Shrimps can survive at low salinities owing to its high euryhaline behaviour and at any given low salinity the ionic makeup of the water is critical for growth and survival and not the salinity itself. Shrimps cannot be reared in salt solutions made of a single salt i.e. NaCl, though Na<sup>+</sup> and Cl<sup>-</sup> are the major ions in seawater. Research has shown that at any salinity i.e. low or high tolerable by the shrimp species the levels of K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup> in the water determine the growth and survival of the species. Rather than absolute levels of these ions, it's the ratio between these ions which determine the feasibility of the low salinity water for shrimp farming. In any inland saline water given the levels of all cations and anions, the levels of K<sup>+</sup> determine survival of the species. As mentioned earlier, saline groundwater from India and Australia have been observed to contain excess levels of Ca<sup>2+</sup> and Mg<sup>2+</sup>, though the Mg<sup>2+</sup>/Ca<sup>2+</sup> ratio is much less compared to seawater due to relatively excess amounts of calcium in saline groundwater. Low salinity inland water in these countries also has an excess of calcium making it suitable for high intensive farming by addition of magnesium salts. However, low salinity inland water in most other countries lack in both Ca<sup>2+</sup> and Mg<sup>2+</sup> and thus calcium also need to be supplemented along with magnesium. Addition of magnesium salts in countries like India and Australia is to bring the Mg<sup>2+</sup>/Ca<sup>2+</sup> ratio to greater than 2.

### **Potassium (K<sup>+</sup>)**

Potassium is generally lacking in most saline water of inland origin. Potassium levels of inland low salinity water are often ten times low as that seen in seawater diluted to the same salinity. High saline operations using saline groundwater generally maintain potassium levels at only 50 % equivalence as that seen in seawater of similar salinity. Potassium is a major intra-cellular cation and is necessary for osmoregulation and maintenance of water equilibrium in all species including shrimp by virtue of the Na<sup>+</sup>/K<sup>+</sup> ATPase activity. The multiplication factor to determine the optimum potassium levels at any salinity is 10.7 which can be used arbitrarily.

For example, a water of salinity 5 ppt shall optimally contain ( $5 \times 10.7 = 53.5$ ) 53.5 ppm of potassium. This is similar to potassium levels in seawater diluted to the same salinity (35 ppt seawater contain about 380 ppm potassium and thus at 5 ppt, diluted seawater shall contain  $5/35 \times 380 = 54.2$  ppm potassium). When *P. vannamei* is farmed in low salinity water close to 10 ppt the aqueous potassium levels are maintained at a minimum of 50% equivalence of seawater of similar salinity. At around 5 ppt, the potassium level for vannamei farming is maintained at 30 ppm and above i.e. greater than 60 % equivalence of that of seawater. However, when farming is carried out at salinities less than 2 ppt, potassium levels are maintained at 100 % i.e. equivalent to seawater of the same salinity.

Potassium in the water is constantly adsorbed by the pond bottom sediments and as the days of culture progresses the aqueous potassium levels drop. At low salinities, higher aqueous levels of potassium are maintained as pond levels can suddenly go down due to adsorption by the pond bottom. Such sudden drop in aqueous potassium levels can affect the shrimp survival and growth at low salinities. At one site near Bhiwani, Haryana the saline groundwater possessed unusually high levels of potassium i.e. >1000 ppm at salinity close

to 10 ppt. Pacific white shrimp PL stocked at this site showed complete mortality in few days due to potassium stress at extremely high aqueous potassium levels.

### **Sodium (Na<sup>+</sup>) and Na<sup>+</sup>/K<sup>+</sup> ratio**

Sodium levels in saline groundwater is not a limiting factor in shrimp farming as its levels are similar or adjacent to Na<sup>+</sup> levels in seawater diluted to the same salinity. However one of the most important parameter overlooked in most low salinity farming operations is the Na<sup>+</sup>/K<sup>+</sup> ratio of the rearing medium. Based on results of experiments in which K<sup>+</sup> alone was provided as the cation source and more than 100 % equivalence of potassium was supplemented to the growing medium, it has been found that rather than the aqueous levels of K<sup>+</sup>, the Na<sup>+</sup>/K<sup>+</sup> ratio of the medium is of greater importance. The Na<sup>+</sup>/K<sup>+</sup>ATPase activity which is vital in osmoregulation is fully dependent on the Na<sup>+</sup>/K<sup>+</sup> ratio of the medium, wherein the enzyme activity lowered with increasing ratio, indicating ratios closer to seawater levels as ideal for shrimp farming. Commercial shrimp farming in low salinity water demands maintaining the Na<sup>+</sup>/K<sup>+</sup> ratio above 30:1 and not more than 60:1 and the ideal range is 30:1 to 50:1. Antony et al. (2015) has found the Na<sup>+</sup>/K<sup>+</sup> ratio between 25:1 and 45:1 as ideal for growing tiger shrimp at 10 and 15 ppt and a Na<sup>+</sup>/K<sup>+</sup> ratio of 25:1 optimum for farming at 5 ppt.

### **Calcium (Ca<sup>2+</sup>) and Magnesium (Mg<sup>2+</sup>)**

Calcium is a major cation essential for shrimp farming as the crustacean exoskeleton development requires calcium to be sequestered from the medium and feed. Calcium along with magnesium also contributes to the hardness of the water and calcium ions prevent large diurnal variations in the water pH due to plankton dynamics as they react with bicarbonate ions HCO<sub>3</sub><sup>-</sup> forming calcium carbonate, CaCO<sub>3</sub>. Calcium is generally not limiting in saline groundwater at salinity 5 ppt to 10 ppt. However, there are reports of calcium deficiency in few saline groundwater's with salinity less than 3 ppt and such deficits are prominent in oligohaline inland water.

Calcium being a critical ion for crustaceans low salinity operations often requires liming to improve Ca<sup>2+</sup> levels. The multiplication factor for determining the optimum Ca<sup>2+</sup> levels at any salinity is 11.6. The calcium levels in low saline shrimp farms should be determined regularly as calcium may be lost in the bottom sediments. Calcium supplementation in low salinity shrimp farming using agriculture lime is not uncommon when required levels are not available in the ground water.

Magnesium is an important cation required for osmoregulation in shrimps. Magnesium is the second most important ion after potassium which needs to be supplemented so as to make the water suitable for shrimp farming. Saline groundwater around the world is mostly deficient in magnesium. Low salinity inland saline waters are deficient in magnesium and thus farming shrimp in low saline waters invariably require supplementation with magnesium salts. Moreover magnesium ions are constantly lost in the sediments and thus regular evaluation of medium magnesium levels is necessary. The multiplication factor to determine the optimum magnesium ion concentration at any salinity is 39.1. Low salinity shrimp farming operations at salinities between 1 to 6 ppt requires the magnesium levels to be over 100 ppm (Roy et al., 2010). Saline groundwater in some parts of the world like India and Australia has higher levels of magnesium compared to seawater

diluted to the same salinity. However, these waters also possess a relatively high calcium levels causing the  $Mg^{2+}/Ca^{2+}$  ratio to drop considerably below seawater levels.

Saline groundwater in India and Australia has an excess of  $Ca^{2+}$  ions in both high and low salinity waters and thus supplementing calcium in these waters is unnecessary. Even with an excess of magnesium, the relatively higher calcium levels define the  $Mg^{2+}/Ca^{2+}$  ratio of these waters to vary from 1.3:1 to 1.7:1 whereas seawater ratios are close to 4:1. Experiences obtained from shrimp farming at Rohtak suggests that, tiger shrimp farming can be carried out without magnesium supplementation as the stocking density is kept at lower levels. However, vannamei farming at stocking densities 60 PL/m<sup>2</sup> or more requires supplementation of magnesium salts in the rearing medium to maintain the  $Mg^{2+}/Ca^{2+}$  ratio higher than 2:1. Therefore in low saline farms where calcium and magnesium are limiting, salts may be added to augment the  $Mg^{2+}/Ca^{2+}$  ratio and low saline farming at salinity less than 2 ppt recommend the  $Mg^{2+}/Ca^{2+}$  ratio to be maintained at >3:1. However in areas where calcium ions are in relative excess supplementation of the medium with magnesium salts is recommended to rise the  $Mg^{2+}/Ca^{2+}$  ratio to over 2:1. This supplementation can be avoided at low stocking densities however at higher densities this procedure is non-avoidable.

## **Conclusion**

Shrimp farming has a bright future in the inland states of the country owing to its unique characteristics and the medium used for farming. The expansion of shrimp farming in Haryana shall open up vistas for other related activities. The high cost of shrimp feed coupled with additional cost on transportation is a major bottleneck for inland shrimp farming. With further expansion in the farming feed mills can be established in Haryana and Punjab so as to reduce the waiting time and the transportation cost. The cost effective vannamei feed "Vannamei+" developed by CIBA, may also be taken up by entrepreneurs in the inland states so as to reduce the production cost of inland shrimp farming. The industrial areas of Gurgaon in Haryana are ideal locations for small start-ups to produce pond blowers, paddle wheel aerators, motors and other farm accessories. Seafood processing plants and large scale cold stores may also be setup in the state so as to promote export trade and local consumption of the commodity.



## **Formulated feed for shrimp farming**

Dr. K. Ambasankar, Dr. J. Syama Dayal, Dr. K. P. Kumaraguru vasagam, K. P. Sandeep and Leesa Priyadarshini and Dr. P. Nila Rekha

### **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  $W \times A$  grams which is equal to  $W \times A/1000$  kg. If feed is to be given at 10% of body weight then the quantity feed required per day is  $(W \times A/100) \times 10/100$  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<sup>0</sup> 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 <sup>2</sup> *	10/m <sup>2</sup> *	5/m <sup>2</sup> *	10/m <sup>2</sup> *
1	0.5	90nil		--	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
20	35.0	70	1.8	1.9	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 inverter 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 be 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.

## Further reading

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# **Diseases and Environmental management**



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

Dr. M. Muralidhar, Dr. S. Suvana and Dr. C. P. Balasubramanian

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 favorable 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	Protozoa	Mysis	Post larvae
Ammonia(NH <sub>3</sub> -N) (µg/l or ppb)	10	17	48	100
Nitrite(NO <sub>2</sub> -N) (mg/l)	0.11	0.29	0.45	1.36
Nitrate(NO <sub>3</sub> -N) (mg/l)	-	-	-	<200
Dissolved Oxygen (%)	-----		>95	-----
H <sub>2</sub> S (µg/l)	-----		<2	-----
Chlorine residue (µg/l)	-----		<10	-----
pH	-----		7.9-8.2	-----
Temperature (°C)	-----		28-32	-----
Salinity ppt	-----		28-34	-----
<b>Metals</b>				
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	-----

**Table 2. Optimum water quality parameters for shrimp aquaculture**

Parameters	Optimum range
Temperature (°C)	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 <sub>2</sub> S (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**

<b>Parameter</b>	<b>Optimum Range</b>
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
Electrical 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<sub>2</sub>S is the key in protecting the losses due to diseases.

## 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 60 $\mu$  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.

## 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. Minimization 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.

## 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,



Fig 1. Source water

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.

### **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.

### **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.

#### Pond preparation

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

#### Draining of ponds

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.

#### 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<sup>2</sup> could be applied. The nitrate salts enhances the organic matter degradation by acting as nitrogen for microbes.

#### 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.



Measurement of soil redox potential

#### 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<sub>3</sub>, quick lime or unslaked lime (CaO), and hydrated lime or slaked lime [Ca(OH)<sub>2</sub>] 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).

#### 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<sub>2</sub>S, NH<sub>3</sub>, CH<sub>4</sub> 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.

- 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 smoothed 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 contact with oxygenated water maintain the oxidized layer.

#### 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<sub>2</sub>S 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.



## **Introduction to disease diagnostics in aquaculture**

Dr. N. S. Sudheer and Dr. Viny T. N.

### **Introduction**

Aquaculture is one of the major economic activities in the tropical and subtropical regions of the world. To meet the dietary needs of the growing population, it is necessary to produce more food. Since the land-based resources are getting depleted over the course of time, now the focus has turned into aquatic farming, as 70% of the earth is covered by water body. Over recent years aquaculture has grown into a multi-billion industry. The growth of the sector is rapid and steady, owing to the latest technological developments which lead to diversification and intensification of the culture. Intensification of the culture, transboundary movements of cultured species as well as frozen fishes and environmental deterioration has led to the introduction and spread of deadly and alien diseases to various nations of the world. Frequent Epizootic outbreaks are major limiting factors of aquaculture production. The situation has led to huge economic losses to fish farmers due to complete crop loss has an adverse effect on the economy of fish farming countries of the world.

Disease management is an integral part of aquaculture Biosecurity measures to facilitate a sustainable fish production. Aquatic animal health laboratories equipped with state of the art facilities and trained personals are now part of any modern aquaculture farms. Disease management strategies in aquaculture are successful only if an effective and early diagnosis of the disease is carried out. Hence, regular monitoring of fish under culture is necessary to reduce the risk of disease onset and spread in aqua farms. Regular monitoring of fishes under culture helps in treating them during the early stages of disease outbreaks as well as to take adequate precautionary measures to prevent the spread of the disease into nearby farms and other water bodies. Diseases that are important to cultured species of fishes and crustaceans are of bacterial, viral, fungal, parasitic, genetic and environment-induced stress related aetiologies.

### **Diagnostics for aquatic animal health management**

To diagnose diseases in commercially important fishes and shellfishes, there are a variety of methods available such as traditional and advanced methods including a diverse range of immunological and molecular methods. Disease diagnosis can be broadly divided into 1. Presumptive (preliminary diagnosis based on gross observations), 2. Confirmatory (Confirmation of the etiological agent with a high degree of diagnostic confidence).

FAO/NACA (2000) defined three levels of diagnosis for aquatic animals as Level I where in gross observation at farm/production site along with record-keeping and health management is carried out. Level II cannot be conducted at farm sit, which requires a laboratory with specializations of parasitology, histopathology, bacteriology, and mycology. At this level, there is a requirement of moderate capital investment and training. Level III comprises various advanced diagnostic techniques which require significant capital and training investment.

Basic principles of diagnostic procedure to detect of presence of etiological agents in a diseased fish, shrimp and mollusc is based on a) traditional methods to assess the

presence of active pathogen (Culturing, electron microscopy etc.) b) testing for the Presence of proteins (immunology based tests)c) nucleic acids (PCR, insitue hybridization etc ).

Aquatic animal diagnosis procedure begins with post mortom observation and generation of basic knowledge on case history, environmental parameters and epidomologicals data. During this stage farmer is asked about details of stocking, feeding and feed and history of disease etc. the information generated at this point of diagnosis is very important in further identification of the cause of death and to decide what type of confirmatory diagnostic procedure to be followed.

### **Traditional methods of diagnosis**

Traditional methods diagnosis involve gross observation, culturing and isolating the pathogen from infected tissue and then identifying the organism involved by biochemical identification (e.g. bacteria), microscopy (e.g. parasites) or electron microscopy (e.g. viruses). Histology and histopathology are also routinely used in disease diagnosis

### **Gross observation**

Gross observation of Clinical signs made at the farm site is very important aid to diagnose and accordingly adequate steps can be taken to reduce the culture lose and prevent the spread of the situation. Mostly observed clinical signs of disease in fish include changes in behaviour, lack of feeding, erratic swimming around the dikes of the pond, unusual aggregations, loss of weight, erratic swimming movement or lethargy, body discoloration, softening of the cuticle, appearance of haemorrhagic lesions, ulcers, and cannibalism. Diseased fishes are often susceptible to parasitic infections. Most of the cases these behavioural changes are accompanied with unusual mortalities. Once these behavioural or clinical signs appear in a population of fish in a pond or aquatic environment, it can be assumed that the fish population is under stress or disease.

### **Microscopy**

Microscopy is a simple and effective tool in fish disease diagnosis, especially for the detection of parasites and fungus. Microscopic examination of skin, gill and internal organs of a diseased fish helps to determine gross clinical signs of disease. Only live or moribund fishes must be taken for microscopy. In case of parasitic infestation, the observation will allow one to undertake remedial measures urgently. Bright field microscopy is a commonly used method. Usually, the specimens are stained before observing. Wet mounts or squash preparation from tissues either stained or unstained are observed. For bacteria commonly used stain is Grams stain, and Grocott's methenamine silver staining technique is done for fungus. Acid-fast organisms such as mycobacteria are visualized using acid-fast stain. To observe unstained, live and transparent organisms, dark field microscopy is used.

### **Electron microscopy**

Electron microscopy allows the visualization of single virus particles as well as cellular organelles. Also, facilitate observation of ultra-structural changes on the cell. An electron microscope works Based on the principle of electron absorption and transmission. Accelerated electron beams are used for illumination of the object, which increases resolution. Through electron microscopy, a Resolution in the nm range ( $10^{-9}$  meters) can be

achieved. There are two types of electron microscopes which differ in their mode of operation and types of the image produced.

**Transmission electron microscopy (TEM):** Its function is based on the principle of electron absorption and transmission. Electrons transmitted through the specimen are detected. Used mainly for observing viruses and cellular organelles.

**Scanning electron microscope (SEM):** It detects secondary electrons which are emitted from the surface of the specimen due to excitation by the primary electron beam. Morphological changes on cells are visualized.

Various technologies are available for visualizing an object in an electron microscope. Negative staining is used for observing purified virus particles. Sodium phosphotungstate or uranyl acetate that will stain background but not the virus particles are used. For tissue samples, tissues are fixed in glutaraldehyde. Staining was done with osmium tetroxide. Processed and embedded in epoxy resin and ultrathin sections are prepared and observed. Shadowing techniques by placing specimen on support and directing a vaporized heavy metal across the sample at an angle. This creates a region where relatively little metal deposits just behind the viral particle (resulting in a shadow).

### **Isolation and identification of pathogenic organisms**

Isolation and identification of causative organisms is a very important step in diagnosing a fish disease. Methods to isolate bacterial, viral, fungal and parasitic organisms from diseased fishes and shellfishes are well established. It is important to use live or moribund shrimp or fish for isolation of pathogenic organisms. Most suitable organ for isolating the pathogen from fish is kidney, and for shrimp it is hepatopancreas. For isolating bacteria in general variety of general purpose media and selective isolation media such as nutrient agar (Freshwater) and Zobell marine media (for marine and brackish water) are widely used. For isolation of specific bacterial pathogen, selective isolation media is used. Accordingly, for vibrio isolation, TCBS is used. For marine and brackish water bacteria addition of 1.5% NaCl is required. Commonly used selective isolation media for various fish bacteria are Rimler-Shotts (*Aeromonas hydrophila*), Cytophaga agar (*Flavobacterium columnare*), Lowenstein-Jensen and Middlebrook 7H10 (*Mycobacterium* sp.), MacConkey agar (Enterobacteriaceae), Baird-Parker (*Staphylococcus* sp.) and Ribose ornithine deoxycholate medium (*Yersinia ruckeri*). Samples from the visceral organs, which seldom contain microbes in healthy fish abscesses, ulcers, skin, gut, blood, ascitic fluid are aseptically removed and inoculated into appropriate media. Inoculation can be done either through serial dilutions of the sample and plating on agar plates or streaking onto the agar plates using a sterile inoculation loop. Plates are then incubated; colonies are counted and isolated for further identification through biochemical tests (e.g., API system).

To isolate the viral pathogen from fish, the most suitable method is isolation on to cell cultures. Primary cell culture & Secondary cell culture, Established cell lines and Organ & explant culture are used to isolate viral pathogens. There are more than 200 fish cell lines available for isolation of virus from fishes. Globally ATCC supplies cell lines for isolation of virus and in India National Repository of fish cell lines (NBFGR, Lucknow) maintain fish 50 cell lines. RTG-2 was the first established and widely used fish cell line for isolation of virus from fish. Commonly used cell lines for isolation of fish virus are RTG-2 (Rainbow trout

gonad), FHM (Fathead minnow larvae), BF-2 (Bluegill fin), BB (Brown bullhead), EPC (Common carp), AS (Atlantic salmon), CHSE-124(Chinook salmon embryo), SSN-1 (Striped snakehead nerve), ASK (Atlantic salmon kidney). Cell culture media such as MEM (Eagles minimum essential media), HBSS (Hanks balanced salt solution) and L-15 (Liebovitz) are used in fish virology. First, a Monolayer of cells is prepared on cell culture flasks or dishes. Then the virus is extracted from infected fish tissue and inoculated into the monolayer and incubated. After incubation cells were observed for the development of Cytopathic effect (CPE). For isolation of crustacean virus's primary cell culture method is used since there were no established cell lines available for isolation of shrimp viruses.

Once the pathogen is isolated from the infected tissue, its pathogenicity should be established by testing Koch's postulate to identify whether the death of the organism is due to the pathogen or other cause. Bioassay is an important method to check the mortality is due to pathogen or toxicity.

### **Histology and histopathology**

Histology involves microscopic observation of thin sections of tissues originated from diseased fishes. Histology is part of routine laboratory technique in any diagnostic laboratory. Specific Pathological changes on tissues due to infection, stress or toxic conditions is explained in histopathology. The technique involves fixing of organs and tissues from moribund fish. Bouins fixative, neutral buffered formaldehyde, and Davidson fluid are some of the fixatives used to fix fish and shellfish. Fixatives are then removed, and tissue is dehydrated in series of alcohol then to xylene and finally embedded in wax. The wax embedded tissue is then cut into thin sections using a microtome. Sections placed on microscopic slides, Wax is removed from the sections and stained in Haematoxylin and eosin. Stained sections were there mounted on DPX and observed on a light microscope.

### **Molecular methods**

Molecular methods for diagnostics make use of modern techniques in molecular biology, requires high investment and expertise. Basics of molecular detection are based on detection of nucleic acids (DNA or RNA). Common molecular diagnostic techniques involve Polymerase Chain Reaction (PCR), In situ hybridization (ISH), Nucleic acid probes, Loop-mediated isothermal amplification (LAMP), Microarray, Whole genome sequencing and Next-generation sequencing.

### **PCR - based diagnostic methods**

Polymerase chain reaction (PCR) was Invented in 1983 by Kary Mullis. Amplification of a region of DNA or RNA which lies between two regions of known sequence is achieved by using specially designed oligonucleotide (18-20 bases) primers which bind to specific DNA sequence. The DNA sequence is copied In the presence of Taq polymerase, dNTPs, and MgCl<sub>2</sub>. Multiple copies of DNA fragment produced. DNA is visualized through gel electrophoresis with the help of UV light after staining with ethidium bromide. PCR based diagnostics is widely used in aquaculture. The advantages of PCR is that it is highly sensitive, high specificity and very effective for virus and bacteria identification.

At present, there are different types of PCR are used in aquaculture

1. One step PCR: Products amplified using a single set of primer
2. Nested or two-step PCR: Two sets of primers used and involve two steps. The product from the first step is again amplified using another set of primer targeting a relatively shorter target. Highly sensitive than one step PCR
3. RT-PCR: Used for RNA viruses. RNA is first converted to cDNA using the enzyme, reverse transcriptase. From the cDNA, the target is amplified using specific primers
4. Multiplex PCR: Two or more primers used in a single reaction. Useful for detecting multiple pathogen infections
5. Real-time PCR: Exact quantification of nucleic acid possible hence, it is highly sensitive than conventional PCR. Suitable for determining virus copy number. Make use of labelled probes and fluorochromes and Level of fluorescence developed after each reaction is measured.

### **In situ hybridization (ISH)**

This technique is first demonstrated by Joseph Gall and Mary Lou (1969). The nucleic acid is localized in the histological section using labelled complementary DNA or RNA (riboprobe) probes. The probes are either directly or indirectly labelled with a fluorophore for visualization. The labelled probe and the target DNA are denatured. Combining the denatured probe and target allows the annealing of complementary DNA sequences. Probes bind with the complementary target sequence and can be visualized.

### **Nucleic acid probes**

Nucleic acid Probes are labelled nucleotides that are complementary to the target sequence. During initial years radioactively labelled probes were used (Eg.P32) for detection of nucleic acids. Recent developments in molecular biology made it possible to use nonradioactive probes (Eg. Digoxigenin (DIG)). The technique involves labeling of probes through Nick translation, random primed labeling, and PCR. Two labelling strategies are commonly used are Indirect and direct probe labelling. In indirect labelling, the probes are labelled with modified nucleotides that contain a hapten. Here an Extra step is required for visualization of the nonfluorescent hapten that uses an enzymatic or immunological detection system. Indirect labelling offers the advantage of signal amplification. Direct labelling depends on nucleotides that have been directly modified to contain a fluorophore. Fluorescent in situ hybridization (FISH) is a faster technique which uses directly labelled probes.

### **Loop mediated isothermal amplification LAMP**

Notomi et al. (2000) of Eiken Chemical Co., Ltd. Japan described the Loop mediated isothermal amplification (LAMP) for diagnostic application. Single tube technique for the amplification of nucleic acids under isothermal condition. Unlike polymerase chain reaction, the target sequence is amplified at a constant temperature of 60 - 65 °C. 2-4 sets of primers are used for amplifying six regions. The DNA polymerase used is Bst DNA polymerase isolated from *Bacillus stearothermophilus*. The quantity of magnesium pyrophosphate precipitate is determined through photometry or turbidity measurement. SYBR green can be

used for visualization and detection. Comparatively more resistant to PCR inhibitors than conventional PCR. Low cost, no need of thermal cycler and high amplification efficiency.

### **Microarray (Biochip or DNA Chip)**

Microarray technique is a kind of hybridization technique in which microscopic spots of a collection of DNA on a chip is prepared. Each DNA spot contains picomoles specific DNA sequence. Fluorescently labelled Target DNA is hybridized to its complementary sequence under a stringent condition. Signal, from a spot, depends upon the amount of target sample binding to the probes present on that spot. The intensity of the signals can be read with the help of a detector and the data generated is analysed with the help of a computer.

### **Whole genome sequencing**

The whole genome of bacteria or virus or any other organism determines the complete DNA sequence of an organism's genome at a single time. Sequencing of an organism's chromosomal DNA, as well as DNA contained in the mitochondria, is carried out. First used technology was shotgun sequencing technology. Under the shotgun method, DNA is broken into numerous tiny segments. The segments are sequenced using the chain termination method to obtain reads. Multiple overlapping reads for the target DNA are obtained by repeating this process. With the help of computer programmes, sequences are assembled, and the genome analysed.

### **Next generation sequencing**

Sequencing of DNA and RNA much more quickly and cheaply than the previously used Sanger sequencing is possible. Various high throughput genome sequencing technological platforms are available to sequence genome rapidly and accurately. Illumina (Solexa) sequencing, Roche 454 sequencing, Ion torrent: Proton / PGM sequencing, SOLiD sequencing are various Next generation sequencing platforms.

### **Immunological methods**

Immunological techniques are widely in use for aquaculture health management are Antibody based techniques that are highly sensitive and easy to operate are based on antibody based commonly used antibody based techniques in aquaculture include Enzyme linked immunosorbent assay (ELISA), Immunohistochemistry, Fluorescent antibody technique (FAT) and Dotblot assay.

### **Monoclonal and polyclonal antibodies**

Antibodies are immunoglobulin molecules that specifically bind to an epitope of an antigen. Based on their origin antibodies are classified into Monoclonal and polyclonal antibodies. Either monoclonal or polyclonal antibody is used in most of the immunodiagnostic methods. Polyclonal antibodies are secreted by B-cells in the body and are multiple cell origin. To produce polyclonal antibody an antigen is injected into animals such as a mouse, rabbit or goat. Usually, an adjuvant is also injected along with the antigen. The injection induces the B-lymphocytes to produce IgG immunoglobulins specific for the injected antigen. Subsequently, after repeated injections and over a period the animal is bled, and the blood is collected. The antibody is purified from the serum.

Monoclonal antibodies (mAb) are a single ancestral cell origin produced by fusion of antigen-specific plasma cells (Produce antibody) with myeloma cells. Georges Kohler and Cesar Milstein 1975 first developed the technique to produce monoclonal antibodies. A hybrid cell (Hybridoma) that produce antibody is developed by fusion of spleen cell and myeloma cells using myeloma cells. The HAT media that contain hypoxanthine, aminopterin and thymidine selectively grow hybridoma cells. Unfused myeloma cells devoid of hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) are unable to grow on HAT media. The hybridoma cells are harvested, and the antibody is purified. Monoclonal antibodies are highly specific.

### **Enzyme linked immunosorbent assay (ELISA)**

The Enzyme linked immunosorbent assay (ELISA), or Enzyme immunoassay (EIA) is a plate based technique to detect proteins, peptides antibodies and hormones in a sample. The antigen to be detected is immobilized on ELISA plate surfaces. As next step, an antibody linked with an enzyme is complexed with the antigen. The conjugated enzyme is then incubated with its substrate. The reaction results in the production of a colored end product. The product can be detected and measured. Commonly used enzymes and substrates in Elisa are Alkaline phosphatase -p-nitrophenyl phosphate, Horseradish peroxides - O-pheylenediamine (OPD) or tetramethyl benzedene (TMD), Beta galactosidase - O-nitrophenyl beta-D-galactopyranoside. Direct ELISA, Sandwich ELISA, and Competitive ELISA are different types of ELISA.

#### **Direct ELISA**

In this form of ELISA, the Enzyme labeled primary antibody directly interact with the target antigen. The substrate is added, and the product is detected after incubation. The technique is easy and simple. Since all proteins in the sample, including the target protein bind to the plate, higher background noise is produced. Hence the direct ELISA is less sensitive.

#### **Sandwich ELISA**

The sample containing an antigen to be detected binds to a plate surface previously coated with a capture antibody. Subsequently, the detecting antibody is added which may be either enzyme conjugated or not. If the detection antibody is enzyme conjugated the technique is referred to as direct sandwich ELISA. If the detection antibody used is unlabelled one more step is required for detection. A secondary enzyme-conjugated detection antibody is used which binds to the antigen, and the technique is known as an indirect sandwich ELISA. In both direct and indirect sandwich ELISA substrate is added and incubated to produce a detectable product.

#### **Competitive ELISA**

Competitive ELISA is used to measure the concentration of an antigen or antibody in a sample. Highly sensitive but very complex to perform. A multiwell plate is first coated with a known antigen. Sample containing the antigen to be detected is applied. Followed by this a labeled detection antibody (Secondary antibody) is applied. Substrate added and signals recorded after incubation. The intensity of the signals depends upon the concentration of antigen in the sample. High concentration of antigen results in reduced signal intensity whereas low concentration of antigen result in little reduction in signal.

## **Immunohistochemistry**

Immunohistochemistry is a combination of histological techniques and antibody based immune assay. Using this technique antigen can be visualized on tissue sections prepared from organs or part of organs from a diseased fish. Infected tissues were fixed, paraffin sections were prepared and mounted on glass slides using standard histology protocols. Antibody solution is added on to the slide and incubated. Further, an enzyme labeled anti-antibody added followed by addition of the substrate to produce a colored product. The slides are then washed and counterstained with haematoxylin and eosin. The slides were then rehydrated, mounted and observed under a light microscope for detection of development of colour due to enzyme substrate reaction.

## **Fluorescent antibody technique (FAT)**

This method is used when looking at the subcellular location of a protein of interest. The basic technique is similar to immunohistochemistry which relays on enzyme labelled antibodies were as the FAT relay on fluorescent labelled antibody and visualization is done on a Fluorescent microscope or a confocal microscope. An antibody or the anti-immunoglobulin antibody is used to detect the antibody labelled with a fluorescent dye, mostly Fluorescein isothiocyanate (FITC) counterstaining was done with 4',6-diamidino-2-phenylindole (DAPI). FAT is classified into two Primary (direct) and secondary (indirect).

## **Dotblot assay**

Simplest, easy, cheapest and widely used diagnostic method to detect an antigen in a sample. The basic principle is the antigen antibody complex formation. Small volumes of antigens or test samples are applied on a protein binding membrane (PVDF or Nitrocellulose). Proteins get immobilized on the membrane. Subsequently, immobilized proteins on the membrane are probed with a specific antibody and a matching specific detection reagent.

## **Importance of environment monitoring in disease diagnosis**

In aquaculture, routine environment monitoring is as important as disease monitoring. This can help in finding the situation that leads to the disease outbreak; accordingly corrective measures can be taken. There is an important relation between culture environment, host and pathogen/parasite. Equilibrium is maintained in between them and any situation that breaks the equilibrium will lead to calamity. The physiochemical variable in a pond ecosystem keeps on change due to human interventions or due to other climatic factors. The major physiochemical variable in aquatic environment includes, dissolved gases, pH, temperature, Salinity (Marine and brackish water), Alkalinity, Hardness, turbidity, Ammonia, Nitrate, Nitrite, and Anion exchange capacity of the bottom soil. First-hand information on physiochemical parameters helps in diagnosing the actual cause of mortality in fishes.

## **Conclusion**

Aquaculture is a significant economic activity that meets the global need of animal protein requirement. The growth of aquaculture is adversely affected by diseases that are



affecting farmed fishes. Disease diagnosis is an integral part of disease management in aquaculture. Aquaculture diagnosis has grown over the period. Molecular and immunodiagnostic methods could be used as a routine diagnostic technique for more sensitivity and specificity. Coupling both modern and traditional methods allow robust, accurate, cheapest diagnostic procedures to detect fish diseases. For effective diagnosis and decision making in fish farms to manage diseases, it is important to understand environmental and physiochemical parameters along with diagnostic test results. Based on this a decision can be taken to go with appropriate management action to undertake.

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## Diseases in shrimp aquaculture - Current scenario in India

Dr. Sathish Kumar T & Dr. S. V. Alavandi

### Introduction

Aquaculture continues to be the fastest growing food production sector with an annual average growth rate exceeding 6 % (FAO, 2014) and having enough potential to meet the growing demands for aquatic food. Aquaculture delivers not only economic income and high quality food products, but also employment to both skilled and unskilled workers. Over the last three decades, shrimp farming has been one of the most rapidly growing aquaculture sectors throughout the world. Shrimp continues to be the largest single seafood commodity by value, accounting for 15% of all internationally traded fishery products. Farm raised shrimp is comprised of 55 % of global shrimp production and this is entirely dominated by two species – the black tiger shrimp (*Penaeus monodon*) and the white Pacific white shrimp (*Penaeus vannamei*) (FAO 2014). In India, shrimp aquaculture started as a traditional practice in natural water bodies such as bheries or pokkali fields and subsequently transformed to commercial industry during 90's. Initially it was dominated by a single species, *Penaeus monodon* and the production of which reached a maximum of 1.44 lakh tones in 2006-07 (www.mpeda.com). Due to disease outbreak and other social issues, the farmers felt it difficult to continue further with this species and thereafter an exotic species; the Pacific white shrimp *P. vannamei* was introduced into the brackishwater aquaculture system of India. Because of its SPF status, fast growth rate and culture feasibility in wide salinity range, this got readily accepted by the farmers and subsequently became the dominant cultured species.

### Significance of shrimp diseases

The increasing trend in intensification and commercialization has exacerbated the epidemics of diseases and become a major constraint for the sustainability of this industry. Severe disease related mortality and thereby economic loss due to different viral agents such as monodon baculovirus (MBV) in Taiwan, infectious hypodermal and hematopoietic necrosis virus (IHHNV) in the Americas, yellow head virus (YHV) in Thailand and Taura syndrome virus (TSV) in the Americas during the different periods have been reported. In addition to all these, the major disease outbreak due to White Spot Syndrome Virus (WSSV) has also been reported from all parts of the world. In India, it is estimated that there is an annual loss of about to tune of Rs. 500-700 crores due to WSD in shrimps. This chapter is aimed at highlighting the most significant and emerging diseases of Indian shrimp aquaculture.

### A. Major diseases

#### 1. Spherical Baculovirosis (*Penaeus monodon*-type baculovirus or Monodon baculo virus [MBV] disease)

#### Aetiology

It is caused by dsDNA virus, PmSNPV (for singly enveloped nuclear polyhedrosis virus from *P. monodon*) belonging to the genus Nucleopolyherdrovirus which was first reported in Taiwan during 1983 and in India during 1995. It is reported that MBV is made up of more than one strain based on its wide geographical and host species range.

### **Susceptible host species**

It is associated with high mortalities in hatchery-reared larval, post-larval and early juvenile stages of *Penaeus monodon*. It infects other penaeid genera or subgenera such as *Penaeus*, *Metapenaeus*, *Fenneropenaeus* and *Melicertus* but in *L. vannamei*, *P. stylirostris* and *P. californiensis* it causes no pathogenesis.

### **Geographical distribution**

It is enzootic in wild and cultured penaeids in the regions of East and South-East Asia, Indian subcontinent, Middle East, Australia, Indonesia, New Caledonia, East Africa, Madagascar, Mediterranean, West Africa, Tahiti, Hawaii, North and South America and the Caribbean.

### **Clinical signs and lesions**

The larval stages (specifically protozoa and mysis) and early PL stages are affected with significant mortalities (from 50% to nearly 100%). Heavy MBV infections in farmed *P. monodon* may suppress growth rate, result in reduced survival and reduce overall culture performance. Protozoa, mysis and early PL stages with severe BP infections may present a whitish midgut (due to the presence of occlusion bodies and cell debris in the faecal material). Diagnosis of MBV infections is made by the demonstration of single or multiple slightly refractive, greenish, intranuclear, spherical occlusion bodies (OBs) in wet mounts of squash preparations of HP or midgut examined by phase-contrast or bright-field microscopy. Staining the tissue squash with 0.05 per cent aqueous malachite green aids in demonstration of the OBs by staining them more intensely than other similar sized spherical objects, such as normal host cell nuclei, nucleoli, secretory granules, phagolysosomes and lipid droplets.

### **Target organs**

It is an enteric virus which can be found in HP or midgut.

### **Disease transmission**

MBV is transmitted horizontally and strictly enteric infecting mucosal epithelial cells of the HP tubules and the anterior midgut.

## **2. White spot disease (WSD)**

### **Aetiology**

It is an acute, highly contagious disease of shrimp, caused by a large, ovoid, bacilliform, non-occluded enveloped dsDNA virus belonging to the family Nimaviridae (Nima: In Latin, means thread) and genus Whispovirus consisting of thread-like polar extension. The virus is inactivated in <120 minutes at 50°C and <1 minute at 60°C and is viable for at least 30 days at 30°C in seawater under laboratory conditions and is viable in ponds for at least 3-4 days.

### **Susceptible host species**

It infects all life stages of decapod crustaceans of marine and brackishwater sources.

## **Geographical distribution**

WSD has been identified from crustaceans in China, Japan, Korea, South-East Asia, South Asia, India, Mediterranean, Middle East and Americas.

## **Clinical signs and lesions**

Clinically, the disease is characterized by lethargy, inappetence, crowded at pond margin, red to pink discoloration of the body, loose cuticle, swelling of branchiostegites, broken antennae, damaged appendages, and the most conspicuous feature of small to large white spots on the inner side of the carapace which spread all over the body in advanced infection. Cumulative mortalities in infected populations may reach 100 per cent within 3 to 7 days of the onset of clinical signs. Histologically, degenerated cells are characterized by hypertrophied nuclei with marginated chromatin and eosinophilic to basophilic intranuclear inclusions.

## **Target organs**

The virus infects ectodermal and mesodermal tissues, especially the cuticular epithelium and subcuticular connective tissues (Eg. pleopods, gills, haemolymph, stomach, abdominal muscle, antennal gland or haematopoietic organ).

## **Disease transmission**

Both vertical and horizontal transmission is reported.

## **3. Infectious hypodermal and haematopoietic necrosis (IHHN)**

### **Aetiology**

The disease is caused by infectious hypodermal and haematopoietic necrosis virus (IHHNV), ssDNA virus of genus *Brevidensovirus* and family *Parvoviridae*. IHHNV is the smallest of the known penaeid shrimp viruses but believed to be the most stable virus of the known penaeid shrimp viruses.

### **Susceptible host species**

Most penaeid species can be infected with IHHNV in all life stages, including the principal cultured species, *P. monodon*, *P. vannamei*, and *P. stylirostris*.

### **Geographical distribution**

At least three distinct genotypes of IHHNV such as Type 1 from the Americas and East Asia (principally the Philippines); Type 2 from South-East Asia; Type 3A from East Africa, India and Australia; and Type 3B from the western Indo-Pacific region including Madagascar, Mauritius and Tanzania are identified so far. The first two genotypes are infectious to the representative penaeids, *L. vannamei* and *P. monodon*, while the latter two genetic variants are not infectious to these species.

### **Clinical signs and lesions**

In *L. vannamei* it causes the chronic disease called runt deformity syndrome (RDS) characterized by lower overall crop production, shrimp with increased size variability and

cuticular deformity. Infected shrimps have been observed to rise to the water surface, remain motionless for a few moments then roll over and sink to the bottom. This behavior may be repeated until mortality occurs. In juvenile *P. stylirostris*, more than 90 per cent mortality reported within several weeks of onset of infection. Gross signs of infection include white to buff mottling of the cuticle, opacity of striated muscle and melanised foci within the hypodermis. In the later stages of infection *P. stylirostris* and *P. monodon* may appear bluish in color. Infected *L. vannamei* display deformed rostrums, cuticle and antennal flagella. IHNV forms Cowdry Type A intranuclear inclusion bodies (IB's) associated with widespread cytopathological changes including hypertrophy of the nucleus and margination of the chromatin in cells of ectodermal (epidermis, gills, hypodermal epithelium of fore- and hindgut, nerve cord and nerve ganglia) and mesodermal origin (hematopoietic tissue, antennal gland, gonads, LO and connective tissues).

### **Target organs**

Tissues of ectodermal (epidermis, gills, hypodermal epithelium of fore- and hindgut, nerve cord and nerve ganglia) and mesodermal origin (hematopoietic tissue, antennal gland, gonads, LO and connective tissues) are the target organs. The enteric organs (endoderm-derived HP, midgut and midgut caeca mucosal epithelia) and smooth, cardiac, and striated muscle show no histological signs of infection by IHNV.

### **Disease transmission**

Transmission can be by horizontal and/or vertical routes.

## **4. Infectious myonecrosis (IMN)**

### **Aetiology**

Infectious myonecrosis (IMN) is a recently identified viral disease caused by dsRNA infectious myonecrosis virus (IMNV), a putative totivirus. IMNV particles are icosahedral in shape and 40 nm in diameter. It is not the same disease as white tail disease (WTD) of penaeid shrimp and *Macrobrachium rosenbergii*. These two diseases exhibit gross and histological signs that mimic IMN, but which is caused by two different types of virus such as a nodavirus named *P. vannamei* nodavirus (PvNV) and *Macrobrachium rosenbergii* nodavirus (MrNV), respectively.

### **Susceptible host species**

It causes mortalities in juvenile and sub adult pond-reared stocks of *L. vannamei* and the mortality range from 40 to 70 per cent. Outbreaks of the disease seems to be associated with certain types of environment and physical stresses (i.e. extremes in salinity and temperature, collection by cast net, etc.), and possibly with the use of low quality feeds. Experimental infection is observed in tiger shrimp, *P. monodon* and blue shrimp, *P. stylirostris*.

### **Geographical distribution**

This disease has been reported in north-eastern Brazil, Java Island, Sumatra, Bangkok, west Borneo, south Sulawesi, Bali, Lombok and Sumbawa in South-East Asia.

There are unofficial and anecdotal reports of IMNV occurring in other South-East Asian countries.

### **Clinical signs and lesions**

Mortalities from IMN can range from 40 to 70 per cent in cultivated *P. vannamei*, and feed conversion ratios (FCR) of affected populations can increase from a normal value of ~ 1.5 up to 4.0 or higher. IMN affected shrimp presents focal to extensive white necrotic areas in the striated (skeletal) muscle, especially of the distal abdominal segments and tail fan, which can become necrotic and reddened in some affected shrimp. By histopathology, shrimp with acute phase disease presents lesions with coagulative necrosis of skeletal muscle. In shrimp recovering from acute disease or those in the more chronic phase of the disease, the myonecrosis appears to progress from coagulative to liquefactive necrosis accompanied with haemocytic infiltration and fibrosis. Significant LO spheroid formation is typically present, and ectopic LO spheroids are often found in the hemocoel and loose connective tissues, especially in the heart lumen and adjacent to antennal gland tubules. In some histological preparations, perinuclear pale basophilic to dark basophilic inclusion bodies are evident in muscle cells, connective tissue cells, haemocytes, and in cells that comprise LO spheroids.

### **Target organs**

IMNV infects tissues of mesodermal origin (striated muscles - skeletal and cardiac muscle, connective tissues, haemocytes and LO tubule parenchymal cells). The enteric organs (endoderm-derived HP, midgut and midgut caeca) show no histological signs of infection by IMNV.

### **Disease transmission**

It is transmitted horizontally by cannibalism and via water. Vertical transmission from broodstock is suspected from anecdotal evidence but it is not known whether this occurs via transovarial mechanism or by surface contamination of newly spawned eggs.

## **B. Emerging diseases**

During the recent past, the havoc created by Early Mortality Syndrome (EMS) in different South East Asian countries has also been found to have severe economic impact on shrimp industry of these regions. Further, a number of other diseases by unidentified etiologies are constantly being associated with shrimp culture practice which has been responsible either for direct mortality or growth reduction and thereby bringing loss to farmers. Shrimp hatcheries are also prone to losses either due to disease outbreak or other unknown factors.

Some of the problems associated with current shrimp culture practice in India are discussed below.

## I. Diseases in Shrimp hatcheries

### 1. Luminescent bacterial diseases

Luminescent bacteria (LB) that cause luminescent disease are ubiquitous to the marine environment and mostly include vibrios. In case of shrimp, this disease is more problematic to hatcheries than the grow-out systems. As vibrios considered as opportunistic pathogens, most of the times the mortality is mainly due to different stress factors caused by poor water quality, crowding, high water temperature, low dissolved oxygen and low water exchange. Different bacterial species recorded to be the cause for luminescent diseases includes mostly of *Vibrio harveyi*, and the rest belong to *V. splendidus*, *V. logei*, *V. fischeri* and *Photobacterium* spp. The main clinical symptoms for this disease are cloudy hepatopancreas, brown gill and body necrosis. Luminescent vibriosis may be controlled in the hatchery by washing eggs with iodine and formaldehyde and avoiding contamination by spawner faeces. *Vibrio harveyi* in the water column can be inactivated by chlorine dioxide. Probiotics are administered directly into the water or via feeds and the immunostimulants successfully used for reducing shrimp mortalities associated with vibriosis. Though antibiotics are efficient to bring good control over the disease, the use of it is not recommended in shrimp aquaculture due to development of drug resistance strains of different bacteria. Biocontrol by bacteriophages can also be used as an alternative to antibiotics. Better Management Practices (BMP), associated with the use of probiotics, immunostimulants and biocontrol agents may be effective ways to control luminescence bacterial disease in shrimp hatchery.

### 2. Zoea II syndrome

In recent years after the introduction of *P. vannamei* in India, Zoea II syndrome causing mass mortalities has been widely reported by the hatcheries. Due to the delayed molting and mortality at zoea II stage, this syndrome was assigned with the name Zoea II syndrome. This was first reported in 1993 from the *P. vannamei* shrimp larvae of Ecuador, Mexico and the United states. *Penaeus stylirostris* (pacific blue shrimp) is the only other species where Zoea II syndrome has also been reported. Generally the zoea II syndrome affected stock looks normal, until the zoea I stage metamorphosed into zoea II. At the zoea II stage, the animals stop feeding, become less active and settle at the bottom. A study done in ICAR-CIBA revealed that, after 36- 48 hrs, of zoea I, the symptoms become evident with the arrest of peristaltic movement, empty gut, no faecal strands and necrosis in the intestinal epithelium. Affected larvae show delayed molting process upto 3-4 days with extreme mortality up to 90 %. The affected larvae were negative for WSSV, IHNV, YHV, MBV, IMNV, HPV, CMNV, and TSV by PCR. *Vibrio alginolyticus* and *V. mimicus* were the predominant *Vibrio* species found to be associated with Zoea II syndrome. Histopathological analysis revealed hypertrophied cells, epithelial cell leakage in to the lumen, vacuolization and disintegration of peritrophic membrane in middle and posterior intestine. The study on the causative agent of zoea II syndrome is limited and the aetiology was poorly understood and documented. Consequently there is no cure for treatment, but restricted stocking in the entire LRT within 3-4 days coupled with better management practices (BMP) will be helpful in prevention of this disease.

## II. Diseases in Grow-out systems

### 1. White faeces syndrome (WFS)

White faeces syndrome reported since last decade, has recently been noted as serious problem for *P. vannamei* throughout the world. However, this disease has been reported from both cultured black tiger shrimp and pacific white shrimp. White faeces syndrome usually occurs after 60 days of culture (DOC) and it may be accompanied by high shrimp mortality. Ponds affected with white faeces syndrome show white faecal strings floating on the pond surface while the shrimps show white/golden brown intestine, reduced feed consumption, growth retardation and often associated with loose shell. The disease can cause moderate to severe economic loss by reducing the shrimp survival by 20–30 percent when compared to normal ponds. While investigating the aetiology of WFS this disease has been associated with presence of vermiform like gregarine bodies, vibriosis, *Enterocytozoan hepatopenaei*, blue green algae and loose shell syndrome. When the contents of the gut or faecal strings were examined in squash mounts with the light microscope, they consisted of masses of vermiform bodies that superficially resembled gregarines. Bacteriological results showed that total bacteria and *Vibrio* spp. found in haemolymph and intestine were significantly higher in diseased shrimp than in healthy shrimp. Histopathological examination revealed diffused haemocyte encapsulation and dilated hepatopancreatic tubules accompanied by necrosis. Tangprasittipap et al., 2013 revealed that the microsporidian newly found in *P. vannamei* is conspecific with previously described *E. hepatopenaei* and it is not causally associated with WFS. Transmission electron microscopy (TEM) study showed that vermiform structures superficially resembling gregarines and commonly found in the HP of cultivated shrimp are not independent organisms but result from the transformation, sloughing and aggregation of microvilli from the HP tubule epithelial cells themselves and the denuded epithelial cells subsequently undergo lysis, can lead to the phenomenon called white faeces syndrome (WFS) and transformed microvilli (ATM) in very severe cases they may retard shrimp growth and may predispose shrimp to opportunistic pathogens (Sriurairatana et al. 2014). Furthermore it has been estimated that the Thai production losses due to WFS in 2010 were 10–15%. The cause of white faeces syndrome and treatment is uncertain. However reduced stocking density, proper water exchange together with better management practices will be helpful in evading White Faeces syndrome (WFS).

### 2. White muscle syndrome

In recent years, shrimp farmers have been suffering from several cases of white muscle with muscle necrosis in the *P. vannamei* grow-out cultures associated with low mortalities. The white muscle syndrome affected shrimps show focal to extensive necrotic areas in striated muscle tissues, displaying a white, opaque appearance. Similar lesions have been described with Infectious myonecrosis (IMN) (Poulos et al, 2006), penaeid white tail disease (PWTD) (Tang et al., 2007) and noninfectious aetiology with sudden changes in water quality parameters such as temperature, salinity and dissolved oxygen. White muscle in shrimp can also be caused by the advanced infection of microsporidians belonging to the genera *Ameson* and *Agmasoma*, or dietary deficiency of selenium. Furthermore, histological analysis from white muscle syndrome affected samples with macroscopic lesions revealed a loss of sarcomeric structure accompanied by coagulative muscle necrosis along with haemocytic infiltration (Melena et al., 2012). Though histological lesions found in the



suspected sample undistinguishable from those reported in *P. vannamei* for IMNV and PWTD there is a small difference in the histopathological change (i.e.) no cytoplasmic inclusion bodies were observed in skeletal muscle of infected samples. Melena et al. (2012) revealed that suspected samples found negative for IMNV and PvNV and suggested that the aetiological agent of this disease could be either a new infectious agent or a different strain of IMNV.

### **3. Running Mortality Syndrome (RMS)**

Since 2011, a new syndrome has brutally affected the shrimp industry and causing substantial mortality. The disease has been loosely termed as Running Mortality Syndrome (RMS) by the farming community. The affected ponds show different mortality patterns with unusual symptoms, no relation to any known diseases and a slow mortality rate (e.g. <1%/day), but the cumulative loss over phase will be high. Some farmers have lost up to four crops, with mortality percentage reaching 70% in most of the cases. Generally mortalities start after a month or 40 days of culture (DOC) but a portion of shrimp continue to survive and can grow to fully harvestable size. Investigations (conducted in ICAR-CIBA) revealed no association of Running Mortality Syndrome (RMS) with known shrimp viral infection. Further, bacteriological examination of haemolymph samples of RMS affected shrimp indicated predominance of *Vibrio* spp., such as *Vibrio parahaemolyticus* and *Vibrio azureus*. The population of anaerobic bacteria in the gut of RMS affected shrimp ranged from 72 - 252 x 10<sup>4</sup> cfu mL<sup>-1</sup> and were identified based on 16S rRNA gene analysis as *Enterococcus faecium*, *E. hirae*, and *Lactobacillus plantarum*. Histopathological examination of the hepatopancreas was largely normal. However, some samples showed karyomegaly and increased inter hepatopancreatic tubular space with haemolymph infiltration, muscle necrosis, loosened LO tubule cells and constricted lumen. Bioassay experiments did not elicit any disease in the experimental shrimp. RMS affected shrimp showed recovery and appeared healthy and active after 155 hrs of transferring to wet lab in water with optimal parameters. Co-habitation experiment with healthy shrimp and the infected animals also failed to induce RMS. All shrimp appeared healthy and active. Relatively few studies done on Running Mortality syndrome, and still the causative agents or aetiology of RMS are unknown.

### **4. Size variation/ Growth retardation**

More recently shrimp farmers have been reported several cases of size variation / growth retardation in *P. vannamei* grow-out cultures. It is reported that viruses, viz., infectious hypodermal and haematopoietic necrosis virus (IHHNV), lymphoid organ vacuolization virus (LOVV), monodon baculovirus (MBV), hepatopancreatic parvovirus (HPV) and Laem-Singh virus (LSNV) are associated with slow growth and size variation in shrimp. In India, Madhavi et al. (2002) recorded multiple viral infections in shrimp with stunted growth. Rai et al. (2009) observed IHHNV, MBV and HPV associated with slow growth shrimp and stated that IHHNV could be one of the causes of slow growth in cultured *P. monodon*. In the event of white faeces syndrome affected animals there is a decrease in feed consumption and growth rates were reduced as revealed by average daily weight gain (ADG) for the whole crop operation of less than 0.1 g/day compared to 0.2 g/day in normal ponds. Feed conversion ratios (FCR) ranged from 1.7 to 2.5 when compared to 1.5 or less for normal ponds (Sriurairatana et al, 2014). Recently *Enterocytozoan hepatopenaei* found to be associated with size variation/growth retardation (Tangprasittipap et al., 2013).

## 5. Hepatopancreatic microsporidiosis

Hepatopancreatic microsporidiosis (HPM) is caused by *Enterocytozoon hepatopenaei* (EHP). It was first reported as an unnamed microsporidian from growth retarded giant or black tiger shrimp *Penaeus monodon* from Thailand in 2004. It was subsequently characterized in detail and named in 2009. It also has much smaller spores (approximately 1 µm in length) and is currently known to infect both *P. monodon* and *P. vannamei*. It has been found that EHP can be transmitted directly from shrimp to shrimp by cannibalism and cohabitation. *E. hepatopenaei* (EHP) is confined to tubule epithelial cells of the shrimp HP and shows no gross signs of disease except retarded growth. It is likely that other penaeid shrimp and/or other crustaceans or even other marine or brackish water species in the region may also be susceptible to infection. For example, some samples of polychaetes and mollusks have tested positive by PCR, but it is still not known whether they are infected or passive carriers. More recently, samples of frozen Artemia mass has been reported to test positive for EHP by PCR, but again, it is not known whether Artemia is susceptible to EHP infection or just a mechanical carrier. It is urgent that these possibilities be explored in order to improve control measures. Although EHP does not appear to cause mortality in *P. monodon* and *P. vannamei*, information from shrimp farmers indicates that it is associated with severe growth retardation in *P. vannamei*. Thus, farmers and hatchery operators monitor *P. vannamei* and *P. monodon* for EHP in broodstock, PL and rearing ponds. The best approach for maturation and hatchery facilities to avoid EHP is not to use wild, captured, live animals (e.g., live polychaetes, clams, oysters, etc.) as feeds for broodstock. Better would be pasteurization (heating at 70°C for 10 minutes). Another alternative would be to use gamma irradiation with frozen feeds. Alternatively, polychaetes could be selected and tested for freedom from shrimp pathogens and then reared as broodstock feed in biosecure settings designed to maintain their freedom from shrimp pathogens (i.e. SPF polychaetes)

## 6. Black gill disease

Black gill disease is very much prevalent in the shrimp farms of Andhra Pradesh. More plankton in water, high stocking density, insufficient aeration and too much mud in the pond bottom is the predisposing factors of this disease. The gill becomes black in colour and is generally colonized with different bacteria (Flavobacterium, Cytophaga, etc.) and parasite (e.g. Zoothamnium spp.). Increase in duration of aeration, water exchange and addition of lime according to pH may be the corrective measures.

## Conclusion

Aquaculture is now integral to the economies of many countries. Growing demand for seafood and limitations on production from capture fisheries will inevitably lead to the increased intensification in commercialization of shrimp aquaculture. This in turn increases the number of diseases and leads to emergence of new diseases. The emergence and spread of infectious disease is usually the result of a series of linked events involving the interactions between the host (including the physiological, reproductive and developmental stage conditions), the environment and the presence of pathogens. Focusing efforts on producing high quality seed, better pond manage to reduce stress and risk of infection, following routine farm biosecurity, responsible trade practices, response to disease outbreak, and improved better management practices shall aid in preventing the epidemics of

diseases. Further health management is a shared responsibility, and each stakeholder's contribution is essential to the health management process.

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*Brackishwater Aquaculture for food, employment and prosperity*



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