Training on

Shrimp culture and disease management in inland saline areas

12-16 December, 2022

ICAR-CIBA TM Series 2022 No. 30

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Training Manual on

SHRIMP CULTURE AND DISEASE MANAGEMENT IN INLAND SALINE AREAS

12 - 16 December, 2022

ICAR-CENTRAL INSTITUTE OF BRACKISHWATER AQUACULTURE

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Preface

Shrimp aquaculture is a lucrative, and continues to be a good agri-business in India with a production of around 1 million tonnes (MT) per annum, most of which is exported, earning a foreign exchange approximating Rs. 46,000 crores. Shrimp farming emerged as a well-established aquaculture activity in India, adopting several technological developments in seed, feed and other farm inputs. Despite all these, there are many challenges for expanding shrimp farming. While shrimp farming is concentrated primarily on the coastal states from West Bengal to Gujarat, inland saline areas in Punjab, Haryana, Rajasthan and western Uttar Pradesh are now emerging as potential areas to expand. Though the basic approaches of farming practices look the same across the diverse farming systems, inland saline areas are unique and demand customization in the farming practices to enhance shrimp production in an economically viable and environmentally sustainable manner. In our understanding, extreme water parameters and their variability across the locations and disease outbreaks remain a major challenge for expanding shrimp culture in inland saline areas. This has helped to utilize saline infested wastelands into profitable commercial use.

Aquaculture is an applied subject requiring much basic scientific research across disciplines. Frequently redefining goals is necessary from time to time based on the prevailing economic situation and technological advancements worldwide. This is only possible through frequent sharing of knowledge and updating on developments in the sector, where training programs could play a major role. At this juncture, it is essential to create awareness among the farming community and state officials about the prospects and challenges of inland saline farming systems.

I am sure this training manual's content is designed to fill the identified gaps in the emerging vibrant farming sector. This would eventually help the farmers and others understand the system as a whole and find customized solutions.

We are incredibly grateful to Dr. J. K. Jena, DDG (Fisheries Science), ICAR, New Delhi for his support, valuable suggestions, and guidance for developing aquaculture in inland saline areas. We also thank the states' ministers, their concerned secretaries and officials for all their support in exploring resources and identifying the issues.

I am optimistic that this training entitled '*Shrimp culture and disease management in inland saline areas*' and this training manual, would very much support the diverse stakeholders of shrimp farming in inland saline areas of Northern India.

All the very best!

KULDEEP K. LAL
Director

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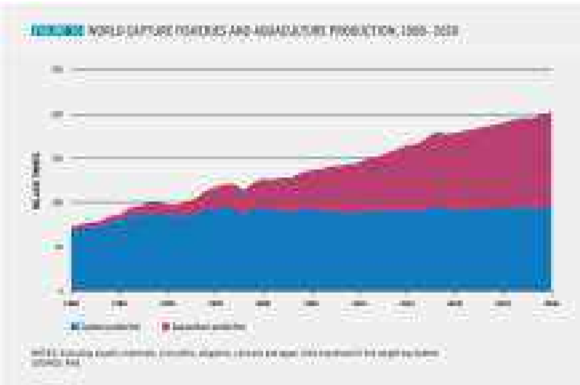
Inland saline aquaculture: A step towards blue transformation

K. P. Jithendran

ICAR-Central Institute of Brackishwater Aquaculture, Chennai

Introduction

With the current rate of population increase, scientists have predicted that the world will need to double its food production by 2050. Aquatic foods are increasingly recognized for their key role in food security and nutrition, not just as a source of protein, but also as a unique and extremely diverse provider of essential omega-3 fatty acids and bioavailable micronutrients. Globally, aquatic foods provide about 17 percent of animal protein, reaching over 50 percent in several countries in Asia and Africa. In 2020, fisheries and aquaculture production reached an all-time record of 214 million tonnes, worth about USD 424 billion. Similarly, the global aquaculture production reached a record 122.6 million tonnes, with a total value of USD 281.5 billion. In 2020, an estimated 58.5 million people were employed globally in the primary fisheries and aquaculture sector and is aimed to increase global aquaculture production from 49% to 53% by 2030 (FAO, 2022). Global consumption of fish has reached 20.2 kg per capita, more than double the average of 9.9 kg per capita in the 1960s.



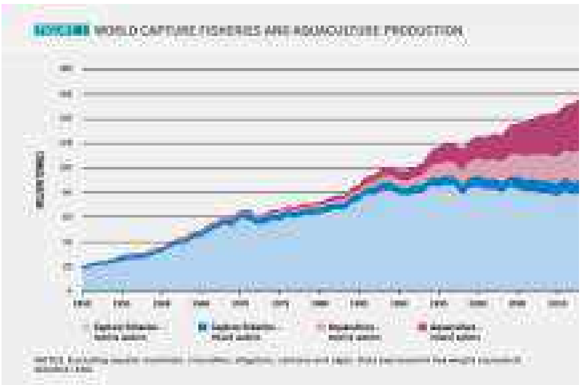
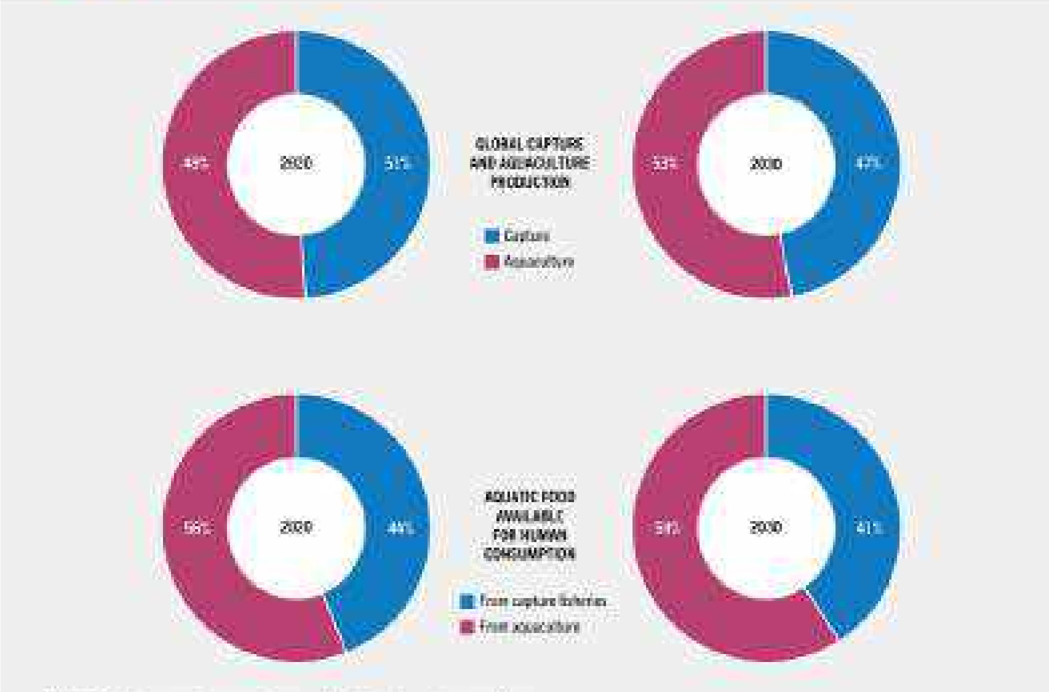


FIGURE 75 INCREASING ROLE OF AQUACULTURE



NOTE: Excluding aquatic mammals, crocodiles, alligators, caimans and algae.
SOURCE: FAO.

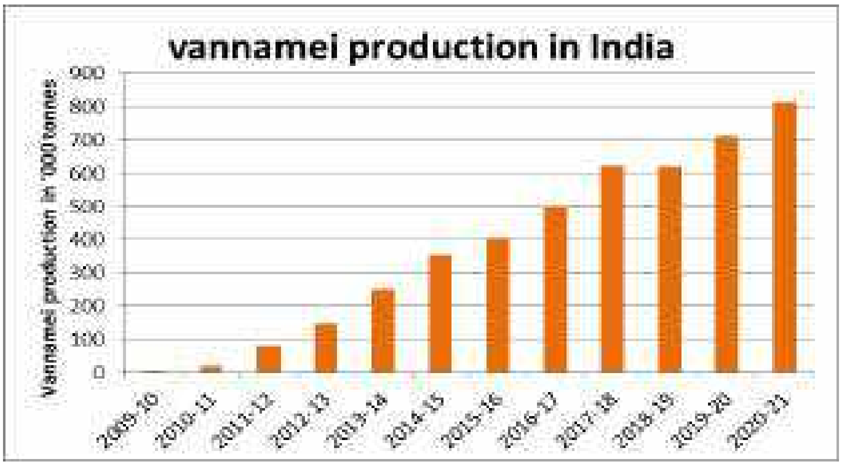
TABLE 18 PROJECTED FISHERIES AND AQUACULTURE PRODUCTION TO 2030

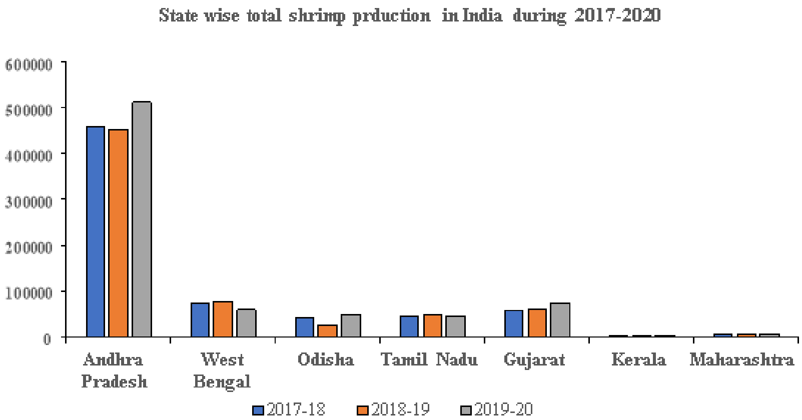
	Production			Of which aquaculture		
	2020	2030	Growth of 2030 vs 2020	2020	2030	Growth of 2030 vs 2020
	1 000 tonnes (live weight equivalent)		%	*1 000 tonnes (live weight equivalent)*		%
Africa	**12 044**	**13 763**	**14.3**	**2 250**	**2 759**	**22.6**
Egypt	2 011	2 339	16.3	1 592	1 911	20.0
Nigeria	1 045	1 208	15.6	262	318	21.4
South Africa	602	522	-13.3	6	12	90.5
Americas	**21 903**	**24 499**	**11.8**	**4 375**	**5 623**	**28.5**
Argentina	840	896	6.7	2	2	10.3
Brazil	1 339	1 527	14.1	629	751	19.3
Canada	901	1 061	17.8	171	244	42.5
Chile	3 259	4 290	31.6	1 486	2 193	47.6
Mexico	1 780	1 910	7.3	279	296	6.2
Peru	5 770	6 210	7.6	144	184	28.2
United States of America	4 694	5 298	12.9	448	548	22.3
Asia	**124 960**	**143 182**	**14.6**	**77 384**	**94 095**	**21.6**
China	62 846	73 608	17.1	49 620	60 068	21.1
India	14 141	16 775	18.6	8 636	10 995	27.3
Indonesia	12 152	13 678	12.6	5 227	6 598	26.2
Japan	3 751	3 471	-7.5	599	684	14.1
Korea, Republic of	1 934	1 933	-0.1	566	633	11.7
Philippines	2 766	3 337	20.6	854	1 045	22.3
Thailand	2 618	2 763	5.5	962	1 113	15.6
Viet Nam	8 023	9 123	13.7	4 601	5 202	13.1

Brackishwater Aquaculture

In India, the brackishwater aquaculture has emerged as sunrise sector, which provide food, nutrition, employment income and livelihood to the lakhs of people in India directly or indirectly. This sector is a significant contributor to the economic development of our country by earning valuable foreign exchange. Indian aquaculture sector has witnessed a 6.5 fold increase in the past two decades and contributed India is endowed with vast areas of brackishwater aquaculture resources amounting to 1.2 million ha, this is beside the 1.24 million ha salt affected soils in coastal areas of about 6.1 million ha inland saline soils, only a fraction of which is currently utilized (over 1.15 lakh ha). These figures highlight the potential and scope for the development of brackishwater aquaculture in our country.

Despite all these efforts brackishwater aquaculture has been largely synonymous with shrimp aquaculture owing to the high export potential of shrimps. The early 1990’s was characterised by a boom in unregulated shrimp farming activities dominated by the giant tiger shrimp *Penaeus monodon.* However, this faced a set-back due to the emergence white spot disease (WSD) caused by white spot syndrome virus (WSSV). Since 1993 WSD has continued to pose a major challenge to the shrimp farming. The consequent economic losses continue to affect the farmers and the shrimp farming sector even today. This has led to the introduction of exotic SPF *Penaeus vannamei* in 2009-10, and in a matter of 5 years, we witnessed a changed scenario of the shrimp farming sector being dominated by white legged shrimp (*P. vannamei*), which has totally replaced the Indian tiger shrimp, a species cultured in 1990’s to 2010. It is estimated that only 11-12% of the potential area is utilized for farming. It is inspiring to witness the spectacular growth of this industry in India, spearheaded by the historical highest shrimp production of 8.44 lakh tonnes in 2021-22 with major contributions from Andhra Pradesh (Figure).





This major species shift took place after a cautious risk analysis undertaken by the government with ICAR-CIBA playing a key role in the introduction of this fast growing species with high export potential. Today again as we take stringent steps to address the potential threat posed by EHP to the shrimp farming sector, we are also vociferous in promoting diversification of aquaculture species and practices. ICAR-CIBA has believed that species diversification in aquaculture and developing need based location specific technologies is the best roadmap for sustainable aquaculture sector for our country.

Inland saline aquaculture

Inland shrimp farming is an attractive proposition that confer several advantages over coastal shrimp farming. The farming being carried out several hundred kilometres from the nearest sea and the medium of farming being pristine ground saline water, the possibility of deadly viral disease outbreaks are much lower. The system is inherently biosecure, though large scale expansion of shrimp farming in these regions requires scientific biosecure practices. The inland regions are also free from mangrove crabs which are a major cause of concern for coastal farms. Coastal shrimp farming have been often criticised to affect highly sensitive coastal ecosystems. Moving farming to inland areas solves this issue as well, as inland areas have more resilient ecosystems.

South Western region of Punjab in India possesses abundant resources of inland saline groundwater with salinities ranging from 3 to 15 ppt. The districts of Bathinda, Muktsar, Fazilka, and parts of Mansa have regions with underlying low saline ground water. Agricultural productivity in this region is lower compared to the rest of Punjab due to low soil fertility, poor irrigation network, the absence of major riverine systems and the presence of saline groundwater. ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA), Chennai successfully demonstrated Pacific white shrimp, *P. vannamei* farming in 2017 using amended inland low saline water in partnership farming mode. We found that inland shrimp farming using the amended low saline water is an alternate farming technology option, with crop duration of 90-120 days for doubling farmers' income through production of high-value shrimp along with employment generation and societal development.

This successful farming demonstration could be a good model where farmers can make good income compared to agriculture by making use of available saline groundwater with high-quality seed and cost-effective desi feed. These also can double the farmer's income in South Western Punjab. However, the development and expansion of inland saline aquaculture in the state need to be monitored and regulated by state department for better utilization of saline water resource and for preserving potable water resources with involvement of R&D institutes. The present training programme is being targeted to meet this objectives, facilitate a scientist-farmer interactions and to examine the status and issues in inland shrimp farming in the state. There is also a growing demand for the need of aqua zoning to regulate and demarcate the notified shrimp farming areas to further boost shrimp farming through a cluster approach and minimize negative impacts.

Major issues

- Lack of testing laboratories is the single most important issue in inland shrimp farming.
- Poor support from state fisheries departments. Farming is mostly driven by enterprising farmers with support from feed companies/their technicians and some central institutions.
- Officials of state fisheries department are not trained in shrimp farming and have no technical know-how on shrimp farming to guide the farmers.
- Farmers are not trained in basic aspects of shrimp farming and no training facilities exist for farmers in these states.
- Aquaculture is not considered at par with agriculture (electricity, insurance etc).
- New markets attract several minor and major players from the Indian shrimp farming industry in the form of feed and medicine suppliers. Most of these companies have poorly trained manpower and technicians in these states who are actually not capable of driving the industry forward.

- Middlemen issues: Agents and middle men source seed from non-registered hatcheries or poor-quality seed often resulting in farmers receiving poor quality seed and incurring losses.
- Shrimp rates on an average are much lower than rates in Gujarat and AP. Farmers may often find it difficult to get a buyer at short notice unlike coastal states.
- No processing infrastructure exists in the state for processing and export of shrimp.
- Since no CAA regulations exist in the state, exporters cannot setup a plant in these states to export shrimp legally.
- Non availability of all inputs required in aquaculture.
- Non-existence of any regulations in the country to regulate inland shrimp farming to ensure sustainability and minimizing issues to agrarian economies inland states.
- Diseases and water quality issues leading to total loss of shrimp due to poor awareness among farmers.

Major opportunities

- Premium quality large sized shrimp can be produced in these states with greater productivity than coastal regions.
- Disease free water and environment. All major pathogens are introduced one,
- Salt affected water can be utilized effectively through aquaculture.
- It is estimated than the three states together account for approximately 12,000 tonnes of production (Table) and in few years would surpass states like Odisha and Gujarat.

Table: Inland saline areas – A new hub for shrimp production

State	~Area	~Production
Punjab (2021-22)	800 acre	2,000 tons
Haryana (2020-21)	1232.5 hectare	3,120 tons
Rajasthan (2020-21)	~100 hectare	750 tons

Currently, work is in progress at ICAR-CIBA on techno feasibility evaluation of inland saline areas for aquaculture, assessment and culture potential of candidate species, issues in health and environment management, aquaculture inputs etc for the development of environmentally and sustainable farming models in Western UP, Rajasthan, Punjab and Haryana.

Future directions for the development of brackishwater aquaculture

Sustainable improvements in technological aspects of aquaculture will not be achieved unless they are accompanied by strong R&D, proper planning and appropriate policies that address the social and economic environment within which the aquaculture system is placed. To assist in national planning, management and policy decisions in brackishwater aquaculture, precise data on the potential resource, technological backstopping and societal linkage are crucial. In the near future, the following key elements related to brackishwater sector may be focussed with adequate policy and funding support to achieve the projected blue revolution mission.

- Promote species diversification to have more choices of finfish and shellfish in the Indian farming basket and its genetic improvement with selected candidates for judicious utilization of resources.
- Promotion and demonstration of diversified farming systems such as cage culture, RAS, pond-based farming, biofloc based farming, farming systems for inland saline soils, IMTA, poly farming etc, for better utilization of available natural water resources.
- Generation of adequate technologies for hatchery production of seeds and indigenous cost-effective feeds for diversified species and demonstrate the technologies with the active participation of the stakeholder.
- Create and strengthen the marketing channels with adequate infrastructure.
- Nationwide disease surveillance and aquatic animal health management and disease control measures to control the spread of exotic disease and unexpected production losses.
- Promote cooperation of state departments, export promotion agencies, central departments & agencies, R&D institutions for joint action

ICAR-CIBA has made commendable progress in the multi-disciplinary areas of brackishwater farming covering captive seed production, feed development, farming system development, disease diagnostics and health management, genetics and stock characterization, climate-smart aquaculture, community engagements using social science tools and policy interventions through government agencies. This would lead to economically viable, environmentally sustainable, socially acceptable brackishwater farming and, ultimately, a significant increase in the total farmed food production. FAO's commitment for Blue Transformation, a visionary strategy focuses on sustainable aquaculture expansion and intensification, effective management of all fisheries, and upgraded value chains. Hence, proactive public and private partnerships are needed to improve production, reduce food loss and waste and enhance equitable access to markets. Climate and environment-friendly policies and practices, as well as technological innovations, are critical building blocks for Blue Transformation.

References for updates:

1. CAA, 2022. https://caa.gov.in/hatcheries_approved.html
2. MPEDA, 2022. https://mpeda.gov.in/#
3. FAO of UN, State of World Fisheries and Aquaculture, 2022. https://www.fao.org/publications/sofia/2022/en/

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Inland saline aquaculture in India: Status, Issues and the way forward

M. Jayanthi, Jose Antony, R. Aravind, T.N. Vinay, and A. Panigrahi

Crustacean Culture Division

1. Introduction

Increasing health consciousness and growing global population protein requirement have increased the demand for aquatic foods. As the capture fishery production is stagnating, aquaculture has proven its pivotal role in fish production at an annual mean rate of 7.5%. Over recent periods, aquaculture has developed as one of the biggest global food industries and grown to produce 82.1 million tonnes of fish in 2018 (FAO, 2020). India is the second-largest shrimp producing country contributes around 7.1% of global aquaculture production and presently dominated with *Penaeus vannamei* farming. India's export growth is primarily due to the success of brackish water aquaculture of shrimp. The unplanned growth of shrimp aquaculture has accompanied with many environmental concerns about misuse of resources, and conversion of agricultural lands into shrimp farms and salinization of land and water resources. As these environmental issues have raised questions about sustainability, aquaculture nations have brought policy guidelines to regulate the development. The guidelines in major shrimp growing nations are Fisheries policy 2004 in China, Coastal Aquaculture Authority Act (CAA) 2005 in India, Article 36 of the Fisheries Law in Vietnam, Marine and Coastal Resources Management Act 2015 in Thailand, and National Shrimp Policy 2014 in Bangladesh (FAO, 2021).

Besides coastal aquaculture, a shift from capture to culture-based fisheries in inland regions has also paved the way for aquaculture development. However, its potential is yet to be realized in terms of production, resource availability, water quality, environmental sustainability and economic stability. Researches have stated that to produce 1 kg of beef, pork, poultry, and herbivorous species of farmed fish (such as carp, tilapia, and catfish), it takes around 7, 4, 2, and < 2 kg of grains, respectively. Though aquaculture development in inland salt affected lands can provide livelihood options from unproductive lands, the water availability, water quality and treatment of discharged water need to be ascertained before planning.

2. Status of soil salinization

Soil salinity is a guide of the presence of salts in soil and is generally stated as electrical conductivity (EC). Soil salinization is a progression by which there is accumulation of salt concentration in soil to an extent that influences on the agricultural activities, environmental well-being, and socio economics conditions of the regions. The salt-affected soils contain excessive concentrations of either soluble salts or exchangeable sodium or both due to inadequate leaching of base forming cations. The major soluble mineral salts are the cations: sodium, calcium, magnesium, potassium, and the anions: chloride, sulphate, bicarbonate, carbonate, and nitrate. Hyper-saline soil water may also contain boron, selenium, strontium, lithium, silica, rubidium, fluorine, molybdenum, manganese, barium, and aluminium.

Figure 1. View of salt affected lands

Soil salinization is a global environmental issue as it affects around 10% of global food production, particularly in coastal countries, and is expected to be more intense in the future due to climate change scenarios, viz sea level rise impact on coastal areas, rise in temperature and thus increase in evapotranspiration, etc. Precise statistics on the global salt-affected land (SAL) spatial database is not yet developed; various data sources provide different information. Globally, 424 million ha of topsoil (0-30 cm) and 833 million ha (30-100 cm) of subsoil are salt-affected, covering 73% of the global land area in118 countries (FAO, 2021). Studies have shown that SAL area has been increasing across the world from 932.2 million ha to 1,128 million ha. Of the salt-affected regions, Asia stands first (65%), followed by Africa (19%), and Europe (5%). The estimations show that globally US$ 27.3 billion loss of crop production annually due to salt-induced land degradation in irrigated areas.

In India, the saline soils are found mainly in the States of Gujarat, Bihar, Haryana, Rajasthan, Maharashtra, Odisha, Andhra Pradesh, Kerala, Tamil Nadu, Uttar Pradesh and West Bengal. Isolated patches of problem soils are also found in other States.

Table 1 Extent of salt affected lands (‘000 ha) in India

Sr. No.	State	Saline soils	Sodic soils	Total
1.	Gujarat	1680.570	541.430	2222.000
2.	Uttar Pradesh	21.989	1346.971	1368.960
3.	Maharashtra	184.089	422.670	606.759
4.	West Bengal	441.272	0.000	441.272
5.	Rajasthan	195.571	179.371	374.942
6.	Tamil Nadu	13.231	354.784	368.015
7.	Andhra Pradesh	77.598	196.609	274.207
8.	Haryana	49.157	183.399	232.556
9.	Bihar	47.301	105.852	153.153
10.	Punjab	0.000	151.717	151.717
11.	Karnataka	1.893	148.136	150.029
12.	Orissa	147.138	0.000	147.138
13.	Madhya Pradesh	0.000	139.720	139.720
14.	Andaman & Nicobar Island	77.000	0.000	77.000
15.	Kerala	20.000	0.000	20.000
Total	2956.809	3770.659	6727.468	

Source: NRSA (National Remote Sensing Agency) Associates (1996) and Adapted from Arora and Sharma (2017).

The salt-affected soils in India broadly fall in two categories: sodic soils and saline soils. At certain places, with mean annual rainfall around 550 mm, saline-sodic soils are also found in the form of narrow band separating saline and sodic soils, but because their chemical properties and management are almost the same as the sodic soils, they are grouped with sodic soils category. Majority of the sodic soils occur in Indo-Gangetic region of India. They originate primarily due to weathering of rocks and minerals containing high sodium minerals, irrigation with groundwater containing excessive quantities of carbonates and bicarbonates, rise in groundwater table due to introduction of canal irrigation and salt laden run-off from the adjoining areas and un-drained basins. The saline soils are widespread in the canal irrigated arid and semi-arid regions. Table 1 shows the distribution of salt-affected soils in India.

Table 2. State wise presence of salt affected lands (%) in India

State	Sodic soils	Saline soil	Coastal saline soils	Total
Gujarat	14.3	71.2	37.1	32.9
Uttar Pradesh	35.6	1.3	–	20.3
Maharashtra	11.2	10.4	0.6	9.0
West Bengal	–	–	35.4	6.5
Rajasthan	4.7	11.4	–	5.6
Tamil Nadu	9.4	–	1.1	5.5
Andhra Pradesh	5.2	–	6.2	4.1
Haryana	4.8	2.9	–	3.4
Bihar	2.8	2.8	–	2.3
Punjab	4.0	–	–	2.2
Karnataka	3.9	0.1	–	2.2
Orissa	–	–	11.8	2.2
Madhya Pradesh	3.7	–	–	2.1
Andaman & Nicobar Islands	–	–	6.2	1.1
Kerala	–	–	1.6	0.3
J & K	0.5	–	–	0.3
Total	100 (3.78)	100 (1.71)	100 (1.25)	100 (6.74)

Figures in parentheses indicate total area in million ha. Source: Adapted from Mandal et al. (2018).

Soil salinity in India is not a very rare phenomenon. It is a natural phenomenon where soluble salts of ions like Sodium, Potassium, Calcium, and Chlorine accumulate in the soil. In areas of high precipitation, they have washed away. While dry areas are more prone to salinization. Excessive use of fertilizers, uncontrolled irrigation, sea water intrusion, are also some of the factors inducing soil salinization. Inland salinity is a major environmental problem that threatens productive agricultural enterprises, fragile ecosystems, valuable infrastructure as well as roads and buildings.

3. Development of inland aquaculture

Of the 177.4 million tons of fisheries and aquaculture production worldwide, 11.4 million tons and 54.4 million tons production has come from inland capture and inland aquaculture.

Figure 2. Saline aquaculture farms in Rajasthan

Shrimp aquacultures in saline regions are growing at a faster rate. Shrimp farms are growing in north India especially in Punjab, Haryana, Rajasthan and Uttar Pradesh. Though the prawn farming had been introduced in Ludhiana, Jalandhar and Kapurthala districts in Punjab and Hissar states, renewed attention is expected to increase the production. At present, shrimp farming has been promoted in 493 ha area in Haryana with a production of ~3,120 ton with an average productivity of 6.32 ton/ha. Action plan for other states has been formulated to bring in more saline affected area under aquaculture for increasing production and thus productivity. Through engagement with ICAR-CIBA, the Department along with the States/UTs is also exploring sustainable ways for species diversification in brackishwater aquaculture by promoting shrimps, oysters, mussels, crabs, lobsters, sea bass, groupers, mullets, milk fish, cobia, and silver pompano.

Additionally, saline water aquaculture is being promoted to transform 'Waste land to Wet-lands' by increasing aquaculture area from 13,000 ha to 58,000 ha by FY 2024-25. This is to boost the current annual production of 4,331 ton to 1.04 lakh ton while boosting current productivity from ~6 ton/ha to 8 ton/ha. States of Haryana, Punjab, Rajasthan and Uttar Pradesh that have high soil salinity are thus being promoted.

Figure 3. Saline aquaculture farms adjacent to agriculture field

4. Issues and way forward in Inland saline aquaculture

Though inland aquaculture has got a plenty of opportunities for utilizing salt affected and unproductive lands, many issues exists for further development as given below.

- The environmental issues due to unplanned inland aquaculture will bring sustainability issues in other nearby productive ecosystems, hence need to be regulated with proper guidelines and policies.
- Water quality monitoring for shrimp aquaculture and proper treatment protocols before discharge is a major concern. As such, there are no systems followed before discharging water in inland saline regions.
- Present shrimp industry is facing major issues due to high production cost and reduced export market price. Shrimp aquaculture is threatened due to disease outbreak, which made the sector as "high-cost, high-risk" one. The farmers with low resource base will have more challenges to tackle the risky situations.
- There is a need to ensure the disease free good quality seed availability as the seed supply totally depends on hatcheries located in East coast states.

- The lack of bio security in shrimp farming operations will bring the problems to the sector itself; hence monitoring system has to be developed.
- In India, Inland regions like Punjab, Haryana, and Rajasthan need to have infrastructure facilities such as feed manufacturing units, shrimp processing plants etc.
- There has to be the ecological mapping and plan to delineate inland regions suitable for aquaculture without affecting other nearby resources after incorporating environmental laws as well as conditions. Use of advanced spatial tools to delineate suitable regions and zones for inland aquaculture, followed by land leasing policy will make the sector more sensible and responsible.
- The salinity and chemical composition of inland saline waters vary with location, even within the same district. Hence, the location specific species suitability need to be studied.
- There is a need to identify the fish species with the less production cost and domestic market potential to make the sector sustainable.
- Group or cluster farming with state or national aquaculture network to make sure the sustainable development in terms of production, resource use, environmental protection and socio economics.

5. Conclusion

The prospective for inland aquaculture production systems is almost unlimited worldwide. With the growing burdens on drinking water and aquatic coastal places, use of inland saline waters offers a key source for seafood production using otherwise unproductive resources. Location specific planning, species selection, environmental monitoring, better management practices, common discharge water treatment system, cluster farming approach and infrastructure development will make the inland aquaculture sustainable and profitable.

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Pre-grow out protocols in Inland saline aquaculture: Seed selection, Acclimatization and nursery rearing

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Crustacean Culture Division

Inland saline aquaculture is a land-based aquaculture system using saline ground water, and it has been practicing to a reasonable level in several countries such as USA, Australia, Israel and India. Across the world a range of finfish and shell has been evaluated for the aquaculture feasibility: *Lates calcarifer, Sparus auratus, Dicentrarchus labrax, Argyrosomus japonicus*), crustaceans (*Penaeus monodon, Penaeus vannamei, P. japonicus, Macrobrachium rosenbergii*), molluscs (*Saccostrea glomerata*). Although many species have been found suitable for cultivation in this type of system, aquaculture of shrimp, particularly *P. vannamei* is found to be developed to the industrial level. Inland saline aquaculture has started in USA in 1970s, and in Australia in 1980s.The majority of the inland saline waters are found in poorly drained, arid, semi-arid low-lying areas where high salt accumulation has occurred. A total of 380 million ha of land spreading around 20 countries are affected by soil salinization. In India, about 8.62 million ha of land are found to be affected by soil salinity, and almost 2.8 million of land is at Indo Gangetic Alluvial plane. Inland saline areas in various states of India are:

Table 1. State wise inland saline area (ha) in India

State	Area (ha)
Punjab	1,51,000
Haryana	2,32,000
Rajasthan	3,75,000
Bihar	1,53,000
Uttar Pradesh	1,37,000
Madhya Pradesh	1,39,000
Jammu & Kashmir	17,000

These inland-saline affected soils, where agriculture does not have a prominent role but water is abundantly available, could be utilized for raising marine/brackishwater aquaculture crops. Owing to the initial work carried out by the ICAR, and state universities saline water aquaculture has been an emerging aquaculture industry in many northern land locked states. Further, these areas are located at remote areas, and land is cheap, and relatively disease free. However, these waters do not have same ionic profile, as marine/brackishwater. Variation in ionic profile occurs even in waters derived from the same aquifer.

This lecture note provides pre stocking protocols for *Penaeus vannamei* aquaculture particularly seed selection, acclimatization and nursery culture. An outline of biology of penaeid shrimp and a comparison of coastal ecosystem and inland saline ecosystem have been summarized for the better understanding of the topic.

Biology of farmed penaeid shrimps

Penaeid shrimps are one most valuable seafood commodities all over the world. FAO statistics record 14 marine shrimps and prawns species items farmed in 60 countries Pacific white shrimp (*Penaeus vannamei*) and giant tiger shrimp (*Penaeus monodon*) account for, respectively, 91 percent and 8 percent of the Indian farmed shrimp and prawn production. *Penaeus vannamei* 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, in areas where water temperatures are normally >20 °C throughout the year. This species inhabits in tropical marine waters. Adults live and spawn in the open ocean, while post-larvae migrate inshore to spend their juvenile, adolescent and sub-adult stages in coastal estuaries, lagoons or mangrove areas. Males become mature from 20 g and females from 28 g onwards at the age of 6–7 months. *Penaeus vannamei* weighing 30–45 g will spawn 1,00,000–2,50,000 eggs. Hatching occurs about 16 hours after spawning and fertilization. The first stage larvae, termed nauplii, swim intermittently and are positively phototactic. Nauplii do not feed, but live on their yolk reserves. The next larval stages (protozoea, mysis and early post-larvae, respectively) remain planktonic for some time, eat phytoplankton and zooplankton, and are carried towards the shore by tidal currents. The post larvae (PL) change their planktonic habit about 5 days after moulting into PL, move inshore and begin feeding on benthic detritus, worms, bivalves and crustaceans. Aquaculture of this species started in Ecuador and later spreads to USA, and by 2,000 this species has become the most farmed shrimp. The reason for the success of vannamei aquaculture is the availability of genetically improved specific pathogen free stock.

The giant tiger shrimp, *Penaeus monodon*, has been the most farmed shrimp across the world until recently. However, this species has been replaced by *P. vannamei*, and the shift was due to the availability of specific pathogen free and genetically improved stock. It is the largest shrimp reaching a size of 270 mm (body length) and 260 g in weight. This species occurs mainly in south-east Asian waters, and they mature and bred in tropical marine habitats. Larvae, juvenile, sub adults are found in coastal lagoons and estuaries, whereas adults are found in 20-50 m depth. Wild male possess spermatozoa from 35 g onwards, and female becomes gravid from 70 g. Animals generally mates in night shortly after moulting, and male deposit spermatophore (packets of sperm) in female seminal receptacle, the thelycum the modified pocket like structure (it is closed thelycum). After mating, the ovary undergoes maturation, and once oocyte maturation completes female release the eggs. Life history phases of *Penaeus monodon* comprises five phases: Egg, larvae, juvenile, adolescent, sub adults and adults (Table 2)

Table 2. Life history phases of *Penaeus monodon*

Phase	Duration
Eggs	12 h
Larvae	10 days
Post larvae	15 days
Adolescent	4 months
Sub adults	4 months
Adults	10 months

There are three larval stages: Nauplii (five sub stages), protozoea/zoea (three sub stages) and mysis (three sub stages) (Fig-1). Post larvae become stockable in production system after 15-20 days of rearing.

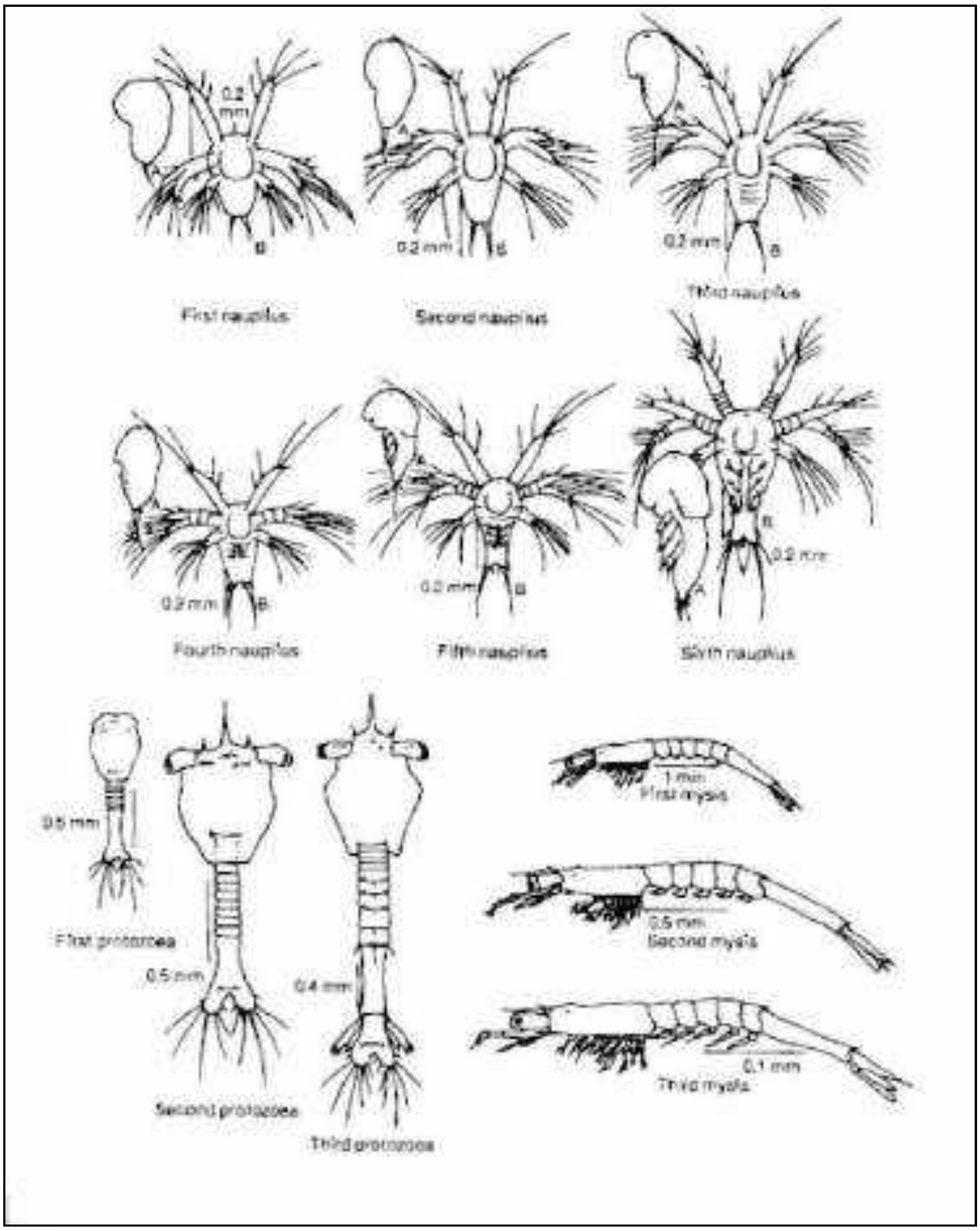

Fig. 1. Larval stages of *Penaeus monodon*

Coastal ecosystem vs inland saline ecosystem

Penaeid shrimps inhabits in coastal and marine waters ranging from salinities 1 ppt to 40 ppt. Salinity is some total of ionic compounds dissolved in one kg water. It is sometimes expressed as mg per Litre of dissolved solids (Total dissolved solids or TDS). A lake with salinity above 3 ppt is called saline at which most people start taste salt. Although salinity of coastal/marine waters and inland saline waters are similar, inland saline water differs in ionic profile when compare to sea water.

Table 3. Concentration of salinity and major ions in inland well water and concentration of those variables in corresponding sea water diluted to similar salinity

Variable	Well water	Sea water diluted to corresponding salinity
Salinity (g/L or ppt)	3.2	2.56
Chloride (mg/L)	1982	1410
Calcium (mg/L)	118.2	29.7
Magnesium (mg/L)	5.46	100
Potassium (mg/L)	11.6	27.4
Sodium (mg/L)	1402	77.9

In general, except potassium and magnesium ionic composition, both sea water and inland saline water is similar. Potassium plays an import role in the physiology of aquatic organism.

Seed quality (Primary selection)

Post-larvae for inland aquaculture is invariable sourced from hatcheries locates at the coastal region. Therefore, criteria for selection of seed are similar to coastal shrimp farming. Although there is no accepted method for determining PL quality, larval qualities can be generally assessed following several criteria and methodological tools that can be broadly categorized into: 1) morphology 2) behaviour, 3) survival to stress test, 4) time needed to complete larval metamorphoses, 5) Survival during larval development 6) PL size 7) Screening of PL for major pathogens.

Morphology

In this category large sets of variables have been used such as size, weight, occurrence of deformities, colour, muscle/ gut ratio, gill and digestive system morphology etc. The simplest and widely used criterion is the visual observation of PL. Active PL with dark colour (depending upon the species) is considered to be best for stocking. The PL with clean carapace should be selected and it indicates the animal is growing fast and moulting frequently. Slow growth is indicated by the presence of pathogens and necrosis

Muscle gut ratio

It was reported that the wild PL has a tail muscle generally exceeds their hind gut diameter by a ratio of at least 4:1. Based on this, muscle gut ratio is used widely to assess the PL quality in many hatcheries. The measurement is taken half way between the telson and last abdominal segment. The muscle should completely fill the shell from the gut down to the ventral side. Poor quality PL will often have muscle gut ratio less than 4:1. This method is proved to be very successful

Stress test

Principle behind the stress test is that high resistance of PL to stress may be a security for their growth/survival performance in grow-out ponds. Salinity stress test: This stress test can be applied to PL8 and PL10. The larvae are held at reduced salinity for a period of 4 hours and then mortalities are recorded, and survival rate above 70% is acceptable. Formalin stress test: The methodology involves: Place a predetermined number of PL in seawater containing formalin, and count the number of live PL after 2 h. If the number of live PL is above the expected number (proposed by them) then the PL used are hardier and can be accepted for further stocking

Screening of post larvae for major pathogens

Larval screening for pathogens has become a pivotal part of the larval quality evaluation. Testing for OIE listed pathogens using molecular diagnostics such as Polymerase Chain Reaction (PCR) has become increasingly common. Sometimes false positive and false negative results may be obtained, in these cases the PL may be tested in multiple labs

Acclimatization of post larvae to inland saline waters

Before culturing one must evaluate the suitability of the water through chemical and biological testes. Ionic composition of the water is more important to the salinity. It has been suggested that calcium (Ca), Potassium (K) and Magnesium (Mg) are most important ions for the survival of the shrimp. Among these ions, potassium is found to be the most crucial ion for the survival of the shrimp. In general, water is suitable for shrimp farming if;

- Salinity is above 0.5 ppt
- Levels of Na, Cl, and K are similar to the levels in seawater diluted to the same salinity

- It has a high concentration of Ca
- Alkalinity should be more than 75 mg/L

Sea water with 35 ppt salinity has 0.38 g/L (ppt) potassium therefore well water with 4 ppt the desirable level would be : 0.38/35 × 4 = 0.43 g/L or 43 mg/L. Another way of calculating the level of various minerals is by multiplying the following factors corresponding to the ions with desired salinity.

Calcium	11.6
Magnesium	39.1
Potassium	10.7
Sodium	304.5
Chloride	551.0
Sulphate	78.3

To amend the inland pond water similar to the coastal brackishwater pond following agricultural fertilizers can be used.

Table 4: Characteristics of mineral salts used in the inland shrimp culture ponds

Mineral salt	**Formula**	**Common trade name**	**Typical composition**
Calcium sulphate	$CaSO_42H_2O$	Gypsum	22% Ca53%SO_4
Potassium Chloride	KCl	Miurate of Potash	50%K; 45% Cl
Potassium Magnesium Sulphate	$K_2SO_4.2MgSO_4$	K-mag	17.8%K; 10.5%Mg; 63.6% SO_4
Magnesium Sulphate heptahydrate	$MgSO_4.7H_2O$	Epsom salt	10% Mg; 39% SO_4
Sodium Chloride	NaCl	Rock salt	39% Na; 61% Cl

To calculate the dose rate of mineral salt for a desired concentration of any one of the variables listed in the last column, use the following equation:

Dose (g/m3) = Desired concentration of variable (mg/L) ÷ Percentage variable in salt/100

For example, if you want to use muriate of potash to increase potassium concentration by 25 mg/L: Dose of muriate of potash = 25 mg K/L ÷ 50% K/100 = 50 mg/L

Post-larvae are procured from hatcheries at the coastal region. The PL must be acclimated to ambient salinities (and ionic profiles) before moving them into grow-out facilities. Acclimation procedures in brackish water or seawater are well established. When PL are transported to inland shrimp farming site, the animals should be shipped not below 15 ppt salinity, as transit mortality is higher in lower salinity shipment.

Two acclimation strategies are commonly used by shrimp farmers. One is temporary holding followed by short-term acclimation (less than 8 hours). The second involves longer holding or nursing followed by a slow acclimation. Short-term acclimation can be used only if the PL are old enough for acclimation, the water source is well suited for culture, and the environmental conditions in the grow-out facility. Nursery systems are required if the PL are too young for acclimation.

Upon receiving PL, the water quality (dissolved oxygen, temperature, ammonia, pH) should be checked in a few randomly selected boxes. Each bag should be inspected to ensure that it is inflated and intact. If the salinity of the receiving water is similar to that of the shipping water (< 4 ppt difference), then only temperature acclimation is required. The most common method is to float the bags in water of the same temperature as the culture water, allowing for a slow temperature acclimation. Once the temperature in the bags is equivalent to the ambient water temperature (1 to 2 hours), the bags can be opened and water added to allow final acclimation to the temperature and ionic composition of the culture water. After acclimation, the PL can be counted, and released. If the salinity of the receiving water differs by more than 4 ppt from the shipping water, the shrimp will need to be acclimated gradually to the salinity of the culture water. This can be done over a relatively short period of time (acclimation) or over an extended period of time (acclimatization). With very low salinity water (< 4 ppt), the longer the period of acclimation the easier the transition will be for the shrimp. It takes 24 to 48 hours for a shrimp to completely adapt to a new salinity.

Acclimation (short duration)

After the bags are inspected, shrimp should be transferred to the acclimation tank and a sample of the PL should be collected and evaluated under a dissecting microscope or visually. PL can be released by emptying the bags. Once the shrimp are in the acclimation tank, new water should be introduced slowly to allow a gradual transition of temperature and salinity. Dissolved oxygen (DO) concentration should be monitored diligently to make sure it remains near or in excess of saturation. As water temperature rises, so will the activity of the shrimp, making the oxygen concentration drop quickly. Temperature changes should not exceed 4 °C per hour. Salinity should not be reduced more than 4 ppt per hour down to 4 ppt. If further reductions in salinity are required they should not be made at a rate higher than 1 ppt per hour.

Nursery culture

The introduction of nursery culture is an emerging concept in shrimp farming in India. The nursery phase for shrimp production was first introduced in 1974, but those time many farmers considered it is not essential and increase the stress to the animal, and most farmers reverted to direct stocking, at present again with *P. vannamei* boom and multiple emerging and new disease threats the use of nurseries is on the rise. Because a nursery phase can intersect the disease transmission and contributes to the rapid growth of the cultured organisms. Nursery system existing in India but was not in much focus till 2016 and since then attention been given to it. In the nursery phase, it is possible to manage higher stocking densities and increase the number of crops per year in shrimp farms

Benefits of Nursery Systems

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%. Shrimp nursery systems can provide several advantages over direct stocking to include: More efficient facility utilization, Greater Bio security, water quality, feed, and feed management, Supply of larger & robust shrimp with high compensatory growth, and overall better survival of shrimp, Decreased grow-out cycle with more crops per year and most importantly serve as primary quarantine period when needed. More over nursery rearing provides compensatory growth to the farmed stock.

In nursery rearing, either cement tank or plastic lined ponds or FRP tanks can be used. PL 10 animals are stocked in higher density (5000 to 10000 PL/mt). Different system such as biofloc, semifloc or mixotrohic system can be used. Typically nursery system varies between 21 to 45 days. Within 30 days Pl reaches about 1 g size with 90% survival.

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Shrimp Farming: Grow-out production and management with special reference to inland shrimp farming using amended saline groundwater

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1. Introduction

Shrimp aquaculture is a major economic activity in several Asian and American countries and and has progressive development. Farmed shrimp is a highly traded commodity, mostly produced in the developing world to sustain markets in the developed countries. However, domestic consumption of shrimp is also increasing significantly in several producer countries mainly due to a rise in income of the working middle class population. White leg shrimp, *Penaeus vannamei* is the most widely farmed penaeid with a production of 5.81 million tonnes in 2020, accounting for more than 84% of the global farmed shrimp production. The production of *P. vannamei* has been following an upward trend with production increasing from 3.8 million tonnes in 2015 to 5.81 million tonnes in 2020. Post 2008, *P. vannamei* was widely introduced to several Asian countries, and the species has gained popularity among the producers and has almost displaced many of the other farmed penaeids in those countries. Giant tiger shrimp *Penaeus monodon* is the second most farmed shrimp globally, with a total production of 0.71 million tonnes in 2020. Unlike *P. vannamei*, the production *P. monodon* is stagnating over the last few years with a production 0.735 million tonnes in 2015 and 0.717 million tonnes in 2020. The proportion of *P. vannamei* in the global farmed shrimp production has increased from 78.8% in 2015 to 84.4% in 2020, indicating an increasing popularity and area under culture for the species. China is the largest producer of *P. vannamei* in 2020 (1.86 million tonnes) followed by India (8.9 lakh tonnes), Ecuador, Indonesia and Vietnam, that constitutes the major producer countries. In 2020, Vietnam (2.63 lakh tonnes) followed by Indonesia (1.33 lakh tonnes) were the largest producers of *P. monodon* globally. India was traditionally a major producer of *P. monodon* until the introduction of *P. vannamei* in 2008, following which the species was replaced by the latter in most farming regions. Shrimp farming in India is principally a pond-based activity in the coastal regions wherein salt affected low lying lands influenced by tidal activity has been traditionally converted to shrimp farms. Shrimp industry in India like any other form of agriculture is ever evolving with farming now been extended further inland to low saline regions (coastal and inland) due to higher pressure on coastal land and greater incidence of viral diseases. Shrimp farming in India is also being carried out in inland states of Haryana, Punjab and Rajasthan, wherein amended inland saline groundwater is used at the rearing medium and presently around 3000 ha has been brought under scientific shrimp farming. Shrimp farming started in North-western India following the pilot scale demonstrations carried out at the Rohtak farm of ICAR-CIFE in Haryana during 2008 for *P. monodon* and 2012 for *P. vannamei* wherein amended inland saline groundwater was successfully used as a growing media for shrimp. Commercial shrimp farming started in Haryana by 2014, backed by subsidy support from the Govt. of Haryana which led to a sudden burst in the number of farms by 2015. Punjab witnessed its first commercial farming activity in 2016 at Rattakhera village of Sri Muktsar Sahib, and on the following year in 2017, ICAR-CIBA carried out the first commercial farming demonstration at Sangat Kalan, Village in Bathinda district of Punjab. Commercial shrimp farming is presently being carried out in Fazillka, Mukstar and Mansa districts of Punjab. Subsequently shrimp farming was demonstrated in Indasar village of Churu District in Rajasthan and shrimp farming has developed in a big way in Rajgarh region of Churu. On the technology side, shrimp farming is evolving from low density farming of *P. monodon* to semi-intensive and intensive farming of *P. vannamei*. Despite intensification in farming activity, shrimp farming in India is still a pond-based activity and 80-90% of the holding are by small and medium farmers who have minimal resources at disposal. The farming is also shifting from direct stocking of seed to two phase and three phase farming wherein nursery reared juveniles are stocked in the place of delicate PL. Intensive farming is performed in geo-membrane lined ponds whereas earthen ponds are employed for low density and semi-intensive farming which constitutes the lion share of production systems for shrimp aquaculture in India. Increasing production costs and emerging diseases have made shrimp farming a high-risk activity, affecting its production and profitability, resulting in a portion of the farming community to return to extensive low-density farming of *P. vannamei* and shift to other candidate shrimp species. The recent introduction of SPF *P. monodon* in India has resulted in significant switch to tiger shrimp farming in traditionally tiger shrimp dominated regions wherein early results suggest excellent come back of tiger shrimp. Since majority of farming in Punjab and the bordering area of Sirsa in Haryana take place in earthen ponds, farming of tiger shrimp can also be taken up in these areas. Technical know-how on shrimp farming is essential for successful management, production and profitability. Sufficient skill sets for management of shrimp farms can be obtained through training and subsequently topped up by the farming experience. Shrimp farmers shall always operate with an element of calculated risk following adequate biosecurity protocols. The following chapter is a brief account on various aspects of shrimp grow-out production management.

2. Shrimp farming production management

Shrimp Farming is science based and technical know-how is essential for successful operation and production. The following section gives a brief account on several key points for successful shrimp farming to the benefit of prospective producers and existing farmers. The information is being presented in a point-to-point form for ease of understanding.

2.1 *Pond preparation*

Pond preparation is essentially carried out to achieve three objectives viz., 1) repair of the pond dikes and bottom, 2) eliminate pathogens from the pond sediments, and 3) facilitate oxidation of organic matter and improve soil pH. The key points to be taken in to consideration while carrying out pond preparations are briefed in the following section.

2.1.1. *Repair of dikes and pond bottom*

- Scrap the sludge and black soil and place them away from the pond instead of elevating them back to the dikes. This procedure can be avoided if sufficient fallow period exists between crops (i.e., bottom soil can be elevated to the dike).
- Following tilling and subsequent elevation and levelling of the soil to the dikes and pond bottom respectively, rotavators or disc harrow shall be employed to mix the top soil.
- Soil placed on the top of the dike and dike slopes shall be compacted using heavy compaction machinery. In the absence of such equipment, the levelling tractor shall be allowed to move over the top and sides of the dike with the leveller fitted in the opposite direction.
- The pond bottom shall be compacted using pneumatic rollers in case of high stocking densities to prevent excessive clay turbidity in the pond during operation of aerators. Farmers, unable to afford a roller, shall fix a wooden log to a tractor via a chain and drag it along the pond bottom to ensure compaction of the sediments.
- In the case of lined ponds, after completion of pond repair and laying of the liners, the liners shall be washed using water at high pressure and all debris scrapped. Following this the liners shall be disinfected by spraying sodium hypochlorite (5-10% Chlorine)

or a solution of 1,000 ppm caustic soda (NaOH), followed by washing with a water jet.

2.1.2. *Elimination of pathogens*

- This step is essential if a confirmed viral disease outbreak i.e., most commonly WSSV occurred during the last crop or shrimp from the previous crop were positive for EHP. These steps are followed simply to target and eliminate the pathogens through disinfection.
- In case of a WSSV infection in the previous crop, dry the pond for 21-30 days which would automatically make the virus non-viable. Additionally, fill the pond to a depth of 1 foot and apply bleaching powder at 100 ppm concentration.
- EHP spores are viable over longer periods and elimination is required to prevent reinfection in the next crop. Elimination of EHP spores is achieved through application of a chemical shock that causes the organism to sporulate and in the absence of a natural host, the parasite follows a natural mortality.
- In the case of EHP spores in the pond sediments, application of hydrogen peroxide (30-35% concentrated) in the dry or wet pond sediments followed by application of bleaching powder after filling the pond to 1 feet water depth is the recommended technique. Alternately, farmers may also resort to application of quick lime, bleaching powder, and caustic soda to eliminate the spores.

- In case of ponds that cannot be dried, or interval between crops are shorts, application of quick lime or hydrated lime at 2 to 3 tonnes per acre would eliminate most pathogens and oxidise the organic matter.

2.1.3. *Oxidation of organic matter*

- Excessive organic matter in the pond sediments from the previous crop when not attended to results in poor pond bottom quality, extremely low ORP values, issues of ammonia and H_2S loading within shorter periods and incidence of black gill disease for reared shrimp.
- Application of lime or hydrated lime immediately post harvesting on the wet soil improves soil pH and enhances the oxidation of organic matter as liming materials improve the soil porosity and keep soil aerated.
- Tilling the pond bottom and allowing it to remain in that condition for one to two weeks significantly reduces organic matter in the soil.
- Intermediate drying and tilling during the pond repair (after removal of top soil), further improves oxidation of organic matter.
- Application of liming material after pond bottom repair followed by operation of rotavators increases the mixing of liming material and top soil, that further enhances the oxidation of organic matter.

2.2. *Water filtration, treatment and disinfection*

The fundamental principle of water filtration, treatment and disinfection is to improve water quality parameters of the source water and thereafter eliminate all pathogens that may otherwise be present in the source water. These steps are inevitable components of scientific shrimp farming and is performed to ensure that the shrimp stocked in to the grow out ponds have the ideal water quality parameters and environment free from diseases causing viruses.

2.2.1. *Water treatment*

- Since inland shrimp farming makes use of saline groundwater that is drawn from aquifers several foot below the surface, water is mostly crystal clear and may not require any filtration.
- However, it should be understood that any water drawn from beneath the soil would have a low pH, high alkalinity, and may have possibly higher levels of ammonia, iron and other heavy metals. Selection of a suitable site for inland saline shrimp farming shall therefore depend on the quality of the bore water available at the site.
- Unlike water drawn from creeks and estuaries which may possess higher organic matter loading and turbidity, saline groundwater generally has very little turbidity and mostly negligible organic matter loading. Hence water treatment practices followed elsewhere for turbidity and organic matter loading can be avoided.
- Ammonia levels in the saline groundwater even if present would gradually nullify as the water is stored in the pond and pH would naturally go up due to exposure to air and sunlight.
- Farmers may however apply EDTA to the pond water at 5 ppm at the beginning of the crop to chelate unwanted heavy metals if present.
- Bore water samples wherein iron content exceeds 0.2 ppm can be avoided and such sites may not be used for inland saline shrimp farming.
- Recommended total alkalinity of the bore water for inland saline shrimp farming is 200-450 ppm wherein source waters on the upper limit can be treated for regulating the total alkalinity within 250-300 ppm. Reduction in total alkalinity for such water can be achieved through agitation of the water and application of highly fermented organic juices, sugars and yeast. Source waters with alkalinity exceeding 500 ppm is not recommended for shrimp farming although water with higher alkalinity can be employed for farming after subjecting them to chemical and organic treatments.

2.2.2. *Disinfection*

- The sole objective of disinfection is to eliminate all pathogens from the intake water and thereby prevent entry of any viruses to the growout ponds. Disinfection is a key step in the pond biosecurity and dictates overall success of the farming operation.
- However, saline groundwater drawn from beneath the soil is technically disease free and is perhaps the greatest biosecurity advantage to inland shrimp farmers. Unlike coastal waters that can be a source for potential pathogens, saline groundwater is free from disease causing pathogens affecting shrimp. Hence, disinfection of the pond water need not be carried out at the same intensity as in the case of coastal shrimp farming. However, farmers should understand the basic concepts of effective chlorination for disinfecting the pond waters prior to fertilisation. Farmers may resort to a minimal chlorination dose of 5 ppm or higher active chlorine concentration.
- Chlorination of saline groundwater also helps in oxidising ammonia and any iron content present in the water.
- The filtration, settlement and treatment steps, would significantly reduce the chlorine demand, such that the applied chlorinating agents can fully eliminate all pathogens, oxidise any remaining organic matter and leave back a free chlorine residue of 1-3 ppm, 24 hours after chlorination.
- Disinfection of water in shrimp farms is generally carried out using stable bleaching powder (grade 1), that contains 30-35% active chlorine. Bleaching powder shall be applied at 10-15 ppm active chlorine concentration in the reservoir and growout ponds during the evening hours.
- The very concept of chlorination is that the quantity of chlorinating agent applied shall fulfil the chlorine demand of the water and retain a free chlorine residual of 1-3

ppm after 24 hours of application. Chlorination at proper doses ensures total disinfection of water and eliminate all pathogens and algae.

- The free chlorine residuals would undergo dissociation in to non-toxic forms under the effect of sunlight and all free and combined chlorine residuals would be lost in 72 hours following which the pond can be fertilised for stocking of seed.

2.3. *Fertilization*

- Application of inorganic fertilisers is not recommended in shrimp farms as they affect the pH of the water. Fermented rice bran (FRB) prepared from super fine rice bran can singly be used to fertilise the disinfected pond water. Single super phosphate may be applied at 1-2 ppm if the bloom fails to develop after repeated application of FRB and chaining, which is generally not experienced in most farms.
- FRB may be applied at 20 ppm concentration as the basal dose followed by a 10-ppm dose after 3-5 days. Another dose at 5 ppm concentration may be applied five days prior to stocking if required.
- Rice bran shall be fermented for a period of 36 to 48 hours using yeast or any other anaerobic probiotic bacteria and applied during the early morning hours. Fermenting rice bran for more than 72 hours is not recommended as it affects the pH of the water. Agricultural lime or dolomite can be added to the FRB mixture 4-6 hrs prior to application to the pond so as to regulate the pH of the solution.
- It is recommended to filter the FRB preparation, using a sieve net and then apply it on the pond surface.
- Following the basal dose application of FRB, chaining of the pond bottom has to be carried out using a heavy chain (30 -50 Kg) for a duration for 2-4 hrs per day for 3-4 days. Chaining ensures release of nutrients trapped in the pond sediments and faster development of bloom. Disturbing the pond bottom using a heavy chain also aerates the pond sediments facilitating oxidation of organic matter and reduced nitrogen and sulphur compounds which in turn reduce the pathogenic vibrio load in the overlying pond water.

2.3.1. *Preparation of fermented rice bran (FRB) [Pond size, 1 acre (4,000m^2), 1m water depth]*

- First dose (20 ppm): 80 Kg of super fine or sieved rice bran preferably raw rice bran, 400-500 litres of freshwater or pond water (1:4 to 1:5 ratio), 10-12 Kg sodium bicarbonate ($NaHCO_3$) and 200 grams of yeast or other anaerobic probiotic bacteria.
- Second dose (10 ppm): 40 Kg sieved rice bran, 160 to 200 litres of freshwater or pond water, 6-8 Kg $NaHCO_3$ and 100 grams of anaerobic probiotic bacteria.
- Third dose (1 ppm to 5 ppm, if required): Adjust quantities as per information given before.
- The mixture is ideally prepared in an FRP tank, or other wide mouth tanks. However, the total capacity of the tank used, shall be 3-4 times the volume of the mixture that is being prepared.
- The mixture shall be aerated continuously using a blower. In the absence of aeration equipment, the mixture shall be frequently agitated using a bamboo or wooden poles. Continuous aeration facilitates fermentation and enables the removal of carbon dioxide formed during the fermentation process. Freshwater is preferred for the fermentation process, although farmers may also the saline pond water.
- Addition of $NaHCO_3$ to the mixture is carried out to prevent, significant reduction in the total alkalinity of the pond water. $NaHCO_3$ increases the alkalinity of the mixture.
- The tank used for preparation of the mixture shall be covered using a plastic sheet.
- The tank is first filled with water to the required levels following which $NaHCO_3$ is added. After the $NaHCO_3$ is dissolved in the water, sieved rice bran is added and mixed thoroughly. Following this preactivated yeast or anaerobic bacteria are added to the water. Even in case the mixture is aerated, intermediate mixing is recommended. The mixture is allowed to ferment for a period of 36 to 48 hours before application. Fermenting the mixture for more than 72 hours is never recommended and such super fermented mixtures should never be applied to the pond as it affects the water quality adversely.

2.4. *Stocking*

- Shrimp seed can be stocked in to the grow-out pond once sufficient bloom has developed in the pond and transparency values reach 30-45 cm.
- Farmers shall check the salinity of the growout ponds before ordering shrimp PL for stocking and the salinity requirements may be communicated to the hatchery in advance.
- Farmers may also analyse the broodstock import data of the hatchery or hatchery conglomerate prior to ordering the PL. The import data is available in the CAA website and farmers may select those hatcheries that have a record of brooder import during the past 4 months.
- SPF broodstock suppliers have developed several genetic lines of *P. vannamei* over the years through selective breeding that exhibits superior growth rate, higher disease resistance or combination of several traits. Famers may decide the strains or lines of their choice based on their requirements, and farming seasons.
- For low saline farming, hatcheries would be able to supply shrimp PL acclimated to low salinities. However, for high saline farming regions wherein salinity is over 32 ppt, seed has to acclimated at the site.
- Check the salinity and pH of the incoming seed and stock the seed post temperature acclimation of 1 hour if salinity difference is less than 3.5 ppt and pH does not vary by more than 0.3. In case of deviations from these conditions, the seed shall be

acclimated to the pond conditions in a FRP tanks and stocked to the pond after 1-2 hours.

- In case of high salinity acclimation, shrimp PL can be acclimated to the growout pond salinity by increasing salinity at the rate of 4 ppt/hour by gradual addition of pond water while providing intense aeration using both air and oxygen. Vitamin C can also be added to the acclimation tank at 25 ppm concentration for best results. Shrimp PL can be stocked to the acclimation tank at a density not exceeding 200 PL/litre. The acclimation tank can be a 1.5 to 2.0 tonne FRP tank.
- Following the same procedure for acclimation before stocking, even under conditions of minor salinity difference, would yield the best survival rate post stocking as it provides adequate acclimation to the pond conditions by minimizing stress. It is always recommended to stock the PL during the evening hours.
- Few shrimps (100-200 nos) should be stocked in to a survival hapa (1 m x 1m x 1m-40-mesh netting) and the number of shrimps shall be counted after 72 hours to roughly estimate the survival at stocking.
- While acclimating *P. monodon*, to high or low salinities, aeration in the acclimation tanks can be maintained gently to avoid stress to the shrimp.
- Shrimp hatcheries often provide additional seed (30-50% bonus), and hence farmers shall regulate stocking accordingly. One or two bags of seed may be counted to estimate the actual number of PL stocked and the stocking density.
- The maximum permissible stocking density for SPF *P. vannamei* and *P. monodon* farming in India is 60 nos./m^2 and 25 nos./m^2 respectively. The stocking density to be followed at a site would depend on the carrying capacity of the pond, experience level and technical knowledge of the farmer, farming season and infrastructure support available at the site. In general, farmers are advised to follow a stocking density of 10 nos./m^2 in case of tiger shrimp farming and 20-30 nos./m^2 in the case of white shrimp. Intensive farming using two-phase or three phase methodology may be practiced by farmers who have larger capital at stake.

2.5. *Feed*

Feed is the most important component in shrimp farming as it accounts for 60% of the production cost and feed management dictates the overall success of the farming operation. Feed is essentially a mixture of several ingredients, so as to fulfil the nutritional requirements of the species. In short, a balanced formulated feed contains the required protein, lipid, carbohydrates, vitamins, minerals, cholesterol and phospholipids for normal growth and metabolism of shrimp. Shrimp feeds are generally formulated using fish meal, soybean meal, krill meal, squid, carbohydrate sources, vitamin and mineral premixes and certain other functional components. Most *P. vannamei* feeds in India has a crude protein level of 35-36%, whereas most *P. monodon* feeds in India have crude protein levels of 38%. Shrimp feeds are marketed in crumble and pellet forms, wherein the crumbled diets are used during the early phase of culture to suit the small mouth size of PL and juveniles, whereas the pelleted diets are employed during the major phase of the culture to feed the advanced juveniles, sub-adults and adults. Some companies offer higher protein diets in the crumble form, i.e., 38% CP for white shrimp feeds and 41-42% CP for tiger shrimp feeds, as few studies suggest that the early life stages have a higher protein requirement.

2.5.1. *Feeding management*

Feed management is the key to shrimp aquaculture and the concept goes viz-a-viz, economics of shrimp farming. Shrimp are fed immediately after stocking in case of high-density nursery and from the next morning of stocking in case of semi-intensive farms.

- Feeding shall be initiated at a rate of 2.0 Kg/1 Lakh seed in the case of semi-intensive and intensive *P. vannamei* farms, whereas initiation of feeding in tiger shrimp ponds can be carried out at a rate of 1.5 Kg/1 lakh seed.
- Crumbled feed shall be broadcasted after wetting them to ensure swift sinking.
- Feed shall be broadcasted 4-5 times a day with an interval of 4 hours between two feeding sessions to ensure proper digestion.
- Feeding during the first month is referred to as blind feeding as farmers are hardly able to see the shrimp and they follow the feeding chart provided by the feed manufacturing company.
- Every feed company gives out a feeding chart for the first month for one lakh seed, following which the feeding rates can be adjusted according to check tray observations, average body weight, climatic conditions and estimated biomass. Feeding rates are also available on the feed bags for ready reference.
- Farmers shall however not follow the feeding chart of the company blindly during the first month and regulate the feed sizes based on shrimp observations. A week after stocking, shrimp can be seen adjacent to the sluice gates or on the leeward side of the ponds on the dike edges during night. By 15 DOC, farmers can start placing check trays and offer some feed to train the shrimp to feed from the trays. Based on these observations farmers can estimate the size of the shrimp and may continue feeding smaller sized feed or shift to the next sized crumble for optimum results. Farmers shall follow the feed quantities in the chart, and feed sizes should be determined on the basis of observations.
- Feeding smaller sized feed for longer durations minimises size variations that can otherwise arise during the culture. However, feeding smaller sized feed to larger sized shrimp would result in clay turbidity, feed wastage and poor growth. Farmers shall strive to strike an optimum.
- As a thumb rule crumbled feeds can be continued until ABW reach 3.5 to 4.0 g following which pelleted diets shall be offered to the shrimp.
- Feeding after 30 DOC shall be strictly on the basis of check tray observations. Ideally a one-acre pond shall be provided with 4 check trays. Feed shall be initially presented at the rate of 2g/Kg feed broadcasted and increased as the ABW increases.

- Always place feed in the check tray after feed has been completely broadcasted in the pond.
- Feeding rates can be adjusted on the basis of check tray observations, ABW, water quality conditions, and estimated survival.
- Feed broadcasting is best achieved through boat feeding wherein feed is spread uniformly in the feeding line. Manual feeding by personnel moving over the dike results in non-uniform feed spreading and feed wastage due to wind action causing some feed to fall on the dikes which can attract birds and crabs.
- Feeding line exists in the aerator swept region few metres from the dikes on all four sides and feed is ideally spread on this line as it is maintained clean by operation of paddle wheels.
- A feeding session or meal can be skipped in case of heavy rain coinciding with the feeding session. Reduce feed by 20-30% during rainy season as lower water temperatures also influence feed intake.
- Place the feed trays gently on the pond bottom to avoid pond bottom mud from entering the tray and covering the feed resulting in false check tray readings.
- Shrimps demonstrating total anorexia may be an indication of disease or deteriorating water quality. If such anorexia is noticed and revival is not seen in 3-4 days, harvesting can be planned.
- Reduce feed by 30-50% during moulting as shrimp reduces feeding activity during pre-moulting and post moulting phases (3-5 days).
- Shrimp feeding on periphyton can be an indication of gut related diseases, vibriosis and under-feeding. Shrimp tends to consume more feed during high temperatures as feeding is expected to rise by 10% for every $1^{o}C$ rise in temperature. Slightly under feeding the shrimp is the best strategy for shrimp farming as over feeding is one of the reasons for gut related diseases.
- No or very few number of shrimps in the check tray after few hours of feeding (check tray observations times are variable with ABW, starting with 3 hours to as low as 1 hour for ABW >25 g) indicates under feeding. Small and broken faecal threads in the check tray indicate under feeding, whereas an excess of black coloured faecal matter indicates that shrimp are underfed and are feeding on live food and debris in the pond.

- Check tray with several shrimp at the time of lifting indicate over feeding. Long faecal threads (feed colour) and individuals with full guts in the tray, indicates over feeding. Long faecal strands are also an indication of shrimp feeding on periphyton that are rich in silica. Feeding rates have to be therefore adjusted according to pond conditions.
- Check trays can be both circular or square shaped with an area of 0.8 to 1.0 m2 and shall be preferably made using stainless steel low weight material and lined using a 40-mesh netting.

Table 1: Feeding strategy based on check tray observations for grow-out pond of size 4,000m^2 equipped with four trays

CHECK TRAY OBSERVATIONS				**Feed adjustment**
Tray 1	**Tray 2**	**Tray 3**	**Tray 4**	
C	C	C	C	Increase feed by 10%
C	C	C	U	Continue same feeding
C	C	U	U	Reduce feed by 20%
C	U	U	U	Reduce feed by 40 %
U	U	U	U	Reduce feed by 50-60%

C=Empty tray (feed fully consumed) U=10-20% or more of lowered feed remain unconsumed

2.6. *Management of soil, water and animal health during production*

Aquaculture is the science of managing soil and water quality in the production system along with management of the animal health. Maintaining ideal soil and water quality in the shrimp ponds ensure proper growth and health status of the reared shrimp.

- Fermented rice bran shall be applied at a rate of 1 ppm twice a week during the first 30 days to sustain the levels of live food organism in the pond water. Subsequently FRB can be applied @ 1ppm every week to manage the C:N ratio in the ponds. As the culture progresses, more nitrogen loading takes place in the pond, that can reduce the C:N ratio. Addition of carbohydrate sources to the ponds regularly i.e., once or twice a week depending on the stocking density, improves the C:N ratio and enhances the growth of heterotrophic bacteria.
- Shrimp farming essentially comprises of two phases i.e., autotrophic phase (0-45 DOC) wherein the major objective is to create green water system dominated by algae and zooplankton providing sufficient live feed to the early life stages of shrimp and a subsequent heterotrophic system (45-120 DOC) dominated by heterotrophic bacteria (brown to golden brown water) to regulate organic matter and nitrogen loading in the shrimp ponds.
- Application of fermented rice bran can create a green water system with sufficient copepods to enhance the growth and survival of freshly stocked PL. In case zooplankton analysis indicates low level of copepods in the water, farmers can apply filtrate of boiled low-cost marine fish at a rate of 20 Kg per acre following the 2nd FRB application and repeat a second dose at half the above rate, 5 days post stocking.
- Addition of carbohydrates sources like molasses or FRB subsequently during the culture, augments the C:N ratio in the ponds and makes the environment conducive for growth of heterotrophic bacteria (brown water system) that can consume organic matter in the pond.
- Application of probiotic bacterial strains shall be performed on a weekly or biweekly basis to improve the water and soil quality parameters. Bacteria belonging to the genus *Bacillus* sp., *Thiobacillus* sp. and *Paracoccus* sp. are effective in reducing organic matter loading in the soil and oxidation of reduced nitrogen and sulphur compounds. Bacteria belonging to the genus *Bacillus* sp., *Rhodococcus* sp., *Rhodobacter* sp., *Nitrobacter* sp., and *Nitrosomonas* sp., are effective in improving

the water quality trough oxidation of ammonia and consume organic matter in the pond.

- Application of these probiotic bacteria can be taken up regularly depending up on the water quality parameters and stocking densities.
- The recommended TAN level in shrimp pond water is 0.25 ppm or lower. TAN levels up to 0.5 ppm may not be dangerous to shrimp, but may affect growth. TAN levels over 1.0 ppm can affect growth and cause disruptions to the immune system and osmoregulatory pathways makings shrimp weak and susceptible to pathogens. At TAN levels over 3.0 ppm, shrimp may exhibit surfacing and anorexia leading to continuous running mortality. To instantly control ammonia, apply sugar, molasses or jaggery at 20 ppm concentration.
- Nitrite toxicity may not be a major issue in high saline shrimp farms, whereas those carrying out operations at less than 5 ppt may be affected by nitrite toxicity. At salinities over 15 ppt, nitrite levels of up to 1-2 ppm does not affect the shrimp negatively and negative effects are only visualised at levels as high as 4.5 ppm which generally do not develop in the ponds. For low saline ponds exhibiting nitrite build-up, especially during the rainy season, nitrite oxidising bacteria can be supplemented to the system to reduce nitrite levels.
- Development of H_2S in the pond bottom sediment is a normal process as the culture progresses and more organic matter accumulates at the bottom. However, it is always beneficial to maintain the soil water interface in an oxidised state to avoid formation of H_2S. To control or eliminate H_2S formed in the pond, aerators can be optimally utilised to drive all sludge to the centre of the pond and subsequently drained through a central drainage system or shrimp toilet. Water exchange in the shrimp pond at regular interval also prevents H_2S accumulation in the pond sediments. Flushing out the bottom layer of the water rich in organic matter is an excellent methodology along with central drainage to regulate H_2S levels.
- H_2S in the pond sediments can also be controlled through probiotic bacteria that are capable of oxidising reduced sulphur compounds. Alternately, H_2S in the pond sediments shall be oxidised by application of peroxide-based chemicals available in granular form. Once the stocking is completed personnel shall never be allowed to enter in to the pond, to avoid disturbance of the pond bottom that can lead to release of underlying H_2S. Exposure of shrimp to H_2S causes loss of appetite leading to

weakness and mortality. Scientific shrimp farming shall make use of a combination of methods to constantly keep H_2S at non-toxic levels.

- pH of the water is the most important parameter for shrimp farming that affects the growth and moulting of shrimp. While pH <7.5 reduces growth, pH more than 8.5 increases susceptibility to viral diseases, inhibits moulting apart from resulting in hard shelled shrimp and black gill disease due to longer inter-moult periods. The ideal pH of water for farming of *P. indicus* and *P. monodon* is 7.5 to 8.0, whereas *P. vannamei* exhibits the best growth at pH levels of 8.0 to 8.3. At low pH, plankton bloom in the pond would be low and water would remain turbid. At higher pH, plankton bloom would be greater and can result in greater diurnal variations in pH.
- To increase pH liming materials can be applied to the water and the application can be continued until the pH stabilises. To reduce pH of the water, application of highly fermented rice bran or rice powder, sugar, molasses and jaggery can be performed. Liming materials such as dolomite or agriculture limestone can also be employed to reduce the pH of the water if diurnal variations are large and plankton bloom levels are very high. In such circumstances application of liming materials during the night can help in precipitation of excess phosphate in the water as calcium phosphate thus controlling the nutrient levels in the pond and subsequently reducing the algal load and pH.
- Alkalinity is the acid neutralizing capacity of the water or the concentration of titratable bases that provide buffering capacity to the water. Alkalinity of the rearing medium shall be 100-120 ppm for the first 30 days followed by 150 ppm for the rest of the culture while rearing tiger shrimp and Indian white shrimp. In the case of white leg shrimp, alkalinity requirements are higher for ideal growth wherein the species performs ideally when alkalinity for the first 30 DOC is around 130-150 ppm and higher levels of 170 to 200 ppm for the rest of the culture.
- Alkalinity can be increased through addition of agricultural lime and dolomite. In case of heavy rains, wherein alkalinity drops significantly, quick lime can also be added to the ponds to sustain the alkalinity levels. While applying quicklime, the liming material can be mixed with water in drum and retained for 24-48 hours before application.
- Experiences from the field suggest that mineral application in shrimp farming may not be required at salinities over 15 ppt. At salinities less than 15 ppt, supplementation of

potassium, magnesium and other trace mineral mixtures (commercial mineral mixes) are applied at 10-20 Kg/acre for optimum growth and moulting of shrimp. At salinities less than 10 ppt, mineral supplementation shall be increased to a level, twice as mentioned earlier. Although mineral supplementation is not necessary at high salinities, mineral bioavailability in the water is also a function of the stocking density, soil characteristics, species and salinity.

- Mineral deficiency symptoms such as cramping and white muscle have even been noticed at high salinities and hyper saline farms (45-60 ppt). Hence farmers can proceed with mineral supplementation based on local observations and experiences. If soft shell is observed in the shrimp integument even during the inter-moult phase, single super phosphate shall be supplemented to the water at 1-2 ppm.
- Gut related diseases are a major issue in shrimp farming. Apart from the etiological agents that may be causing these diseases, over feeding is another major factor that contributes to gut related illness. Avoid over feeding the shrimp to reduce emergence of gut related illness.
- Supplementing the feed with probiotics, vitamins and other nutraceuticals through top coating may be performed if necessary. Such supplementations if required, may be carried out only after 15 DOC and ideally after 30 DOC as shrimp may be hardly feeding on the formulated feed offered during the first 15 days. In a nursery, such additions can be carried out from day 1, as high animal density due to restricted space would ensure feed uptake in a high feed availability scenario.
- Probiotic bacteria shall be activated by immersing the required quantity in freshwater for 3-4 hours and subsequently top coated over the diet for best results as gut transition time of feed is only 1-2 hours.
- Aerators shall be operated optimally during the culture. As a thumb rule, 1HP motor with two paddles is capable of holding 300 kg of biomass in the pond. Increase the number of aerators if the estimated biomass is high and DO levels during the morning are critically low. Place the aerators such that the direction of the flow creates a centrifugal force driving sludge and uneaten feed to the centre of the pond.

3. Conclusion

Shrimp farming is a noble profession and a high-risk high reward business, wherein technical know-how, hard work and constant monitoring of the water quality and animal health bear fruitful rewards. Shrimp farming and its stake holders contribute immensely to the national growth and is a major vehicle augmenting India's export revenues and forex earnings. Increasing shrimp production is key to India's growth and producing more shrimp within a unit area, would be the focus of the future. In a country like India where coastal resources have several conflicting end uses, vertical expansion of shrimp farming is the only way to increase farmed shrimp production and improve the country's export earnings. By 2024-25, India targets to raise its revenue from export of fishery products to a whopping Rs. 1 lakh crore, which necessitates a quantum jump in fish production. Over the years farmed shrimp has spear headed India's export earnings, accounting for more than 70% of the value realized. Therefore, producing more shrimp would be the best way to boost the country's export earnings.

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Diversification of brackishwater aquaculture species and system opportunities in Inland aquaculture with focus on Eco-based farming

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Introduction

Aquaculture is emerging as a new hope to feed the growing population that put thrust on each and every resource to be utilized sustainably for production of food. Inland saline water aquaculture is one of- the important avenues for it as vast unutilised resources are available. Brackishwater aquaculture in India is almost synonymous with shrimp farming. In 1950s it was all traditional farming which is basically the paddy field shrimp fishery which has been evolved into a primitive form of aquaculture where, the naturally immigrating shrimp seeds from coastal waters are entrapped and reared for few months, without any feed or aeration. With time as the demand for shrimp increased as export commodity, shrimp farming further evolved in to selective stocking and feeding with modified extensive farming and thereafter as commercial hatcheries, intensification and high-density farming started. 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. From 2000 to 2006 shrimp farming gradually increased and peaked with a maximum production of about 1.4 lakh tonnes in 2006, but production reduced drastically in 2008. Again, 2009-2020 witnessed a remarkable upsurge of farmed shrimp, due to the introduction of, the exotic American shrimp, *Penaeus vannamei*, resulting in an increased production of about 1,25,000 tonnes in 2012-13 to 6,43,037 MT in 2021-22 (MPEDA). Now India ranks second in global total fish production stands at 16.187 million tonnes (2021-2022) which is about 7.7% of total global fish production (PIB, Govt. of India).

The major inland saline lands of the world are found in arid, semi-arid and low-lying and poorly drained regions, where high concentrations of salts accumulate in the soil. Out of a total area of 6.74 million hectares that are affected by salt (including coastal saline soils) in India, about 1.20 million hectares (or 12 lakh ha) are located in the non-coastal Indo-Gangetic plains in northern India and covering seven states, including Punjab, Haryana, Rajasthan, Bihar, Uttar Pradesh, Madhya Pradesh and Jammu and Kashmir where agriculture does not have a prominent role but water is abundantly available– can be potentially utilized for developing aquaculture.

2. 1. System Diversification:

Over the years, ICAR-CIBA has generated significant information on shrimp, fish, crab hatchery and grow-out production, nutrition and feed-technology, disease diagnosis and management to address the growing needs of brackishwater aquaculture sector and provided a platform for interaction with stakeholders. Generally, the poor health, reduced growth rate and frequent mortality are some of the symptoms of the inland saline aquaculture. Inland saline well waters (ISWW) do not have the same ionic composition as marine or brackish waters but they can be made suitable for survival and growth of marine species, if chemically modulated. Inland saline waters differ from each other and variations in ionic profiles occur even in waters derived from the same saline aquifer. In general, except for low K+ concentration, ionic composition and concentration of ISW is similar to the ocean water. the ionic ratio of inland saline water was varying from sea water and when these ratios were corrected by the fortification of potassium, magnesium and calcium salts the survival of P. monodon was improved .It was reported that complete mortality of *P. monodon* in potassium deficient inland saline water while in potassium supplemented inland saline water survived well at the salinity of 5, 10 and 15 ppt. These technologies have the ecosystem approach-based footprints and are discussed here.

A. Biofloc based technology for brackishwater species

Biofloc technology is a relatively new technology to support high density, better water quality, water conservation, bio security, lower feed requirement and reduce the production cost. The concept of biofloc technology work around the formation of dense heterotrophic bacterial community. Eventually the system becomes bacterial dominated rather than algae dominated and forms microbial flocs by utilizing the waste materials in the pond. Biofloc is the conglomeration of microorganisms (such as heterotrophic bacteria, algae (dinoflagellates & diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans & detritus). Constant aeration and intermittent addition of carbon source as organic matter for the bacteria is needed to prevent the collapse of the system.

In a typical brackishwater pond, 20–25% of fed protein is retained in the fish/shrimp, rest is wasted as ammonia and other metabolites. Manipulating the C:N ratio in the pond enhances conversion of toxic nitrogenous wastes into microbial biomass available as food for culture animals. CIBA has initiated efforts to develop a biofloc model suitable for Indian brackishwater farming systems. A series of experiments in pilot scale was conducted at CIBA showed measurable gain in the production as well as FCR in tiger shrimp *P. monodon* farming by following these eco based techniques. Several studies indicates that bio-floc with periphyton systems (BPT) increased growth, survival and protective response.

Biofloc based Nursery rearing

A nursery system can be defined as the intermediate step between the early post-larval (PL) stage and the grow-out phase especially in shrimp culture. Intensive nursery systems present several benefits such as optimization of farm land, increased shrimp survival and enhanced growth performance in grow out ponds. This phase is decided as an intermediate step between hatchery-reared, early post larvae (PL) and the grow out. Strategies for this phase involve holding postlarvae at very high densities in small tanks for 15 − 60 days with precise technical management, feeding and water quality monitoring. In some countries, e.g., Brazil and Ecuador, intensive nursery (∼ 60 PLs L−1) of short duration has been used to produce 20- or 25-day-old PLs in a two-phase production system. Higher income generation due to higher stocking density in nursery system to achieve higher profitability.

Advantages of biofloc based nursery technology

- Daily water and pond environmental changes (pH, O_2, CO_2) during nursery are minimized for optimal performance.
- Decreased nutrient conversion rate due to continuous consumption of high nutrient feed by autophagy or root microbes.
- Reduced size variability in growth event shrimp compensated by better growth performance.
- Stimulates immune system under these systems, resulting in healthy shrimp.
- It improves productivity, natural feed, FCR, economic gain; and reduced costs (15-20% lower cost production).
- Pathogenic bacteria (Vibrios) are reduced by probiotic intervention and toxic metabolites (NH_3-N, NO_2-N) in the nursery.
- Through the microbial association technology, the nursery shrimp culture system uses twice the number of proteins (feed & converted waste), improving digestion (with enzymes and growth promoters) and increasing growth.

Biofloc-based grower stage shrimp culture system

Biofloc technology is a cutting-edge method of recycling pond waste with carbon sources and probiotics to produce natural feed, which improves pond quality, water quality, and productivity while lowering costs. ICAR-CIBA have standardised the biofloc production protocol for biofloc based grow-out systems, nursery culture, protein levels in feed, carbon source selection, carbon: nitrogen ratio, periphyton substrate selection and applications, and probiotic & non-probiotic shrimp culture systems. In one pond based experiment, higher survival rate was recorded in bio-floc groups followed by SF groups than control. Similarly, ABW, FER, FER and production was significantly ($P < 0.001$) increased in BF groups followed by SF groups when compared to control. The FCR was significantly reduced in the BF and SF groups than control groups ($P < 0.002$).

Growth parameter	**Control**	**Semi floc**	**Biofloc**	**P = value (*)**
Survival rate (%)	$72.87^{a}\pm7.03$	$85.67^{b}\pm3.45$	$95.23^{b}\pm3.25$	0.043
ABW (g)	$22.43^{a}\pm1.01$	$26.43^{b}\pm1.75$	$34.53^{c}\pm2.01$	0.001
FCR	$2.30^{a}\pm0.26$	$1.63^{b}\pm0.15$	$1.33^{b}\pm0.12$	0.002
FER	$0.44^{a}\pm0.06$	$0.62^{b}\pm0.06$	$0.75^{c}\pm0.07$	0.002
PER	$1.25^{a}\pm0.14$	$1.75^{b}\pm0.16$	$2.15^{c}\pm0.19$	0.002
Production (tone/ha)	$10.25^{a}\pm1.75$	$17.71^{b}\pm1.27$	$19.46^{c}\pm2.12$	0.002

B. Organic production system for brackishwater species

Organic aquaculture is a process of production of aquatic plants and animals with the use of only organic inputs in terms of seeds, supply of nutrients and management of diseases. Organic production system is an ecosystem based approach to aquaculture. Organic foods have a separate niche market and many farmers are attracted to these farming practices due to lower cost of production and better economic returns. In India, INDOCERT provides certification for organic production systems. Although organic aquaculture is in a very nascent stage in India, its traditional system is close to the organic way of farming.

C. Organic Aquaculture: Periphyton based farming

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

D. Polyculture based production system

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

E. Integrated multi-tropic Aquaculture (IMTA)

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

F. Bio secured zero water exchange shrimp farming technology (BZEST)

Bio secure zero-exchange system for shrimp represents an emerging technology that provides a high degree of pathogen exclusion with minimal or zero water exchange. This zero water exchange shrimp farming system is an evolving culture practice with use of probiotics and zero tolerance to banned chemicals and antibiotics. CIBA has developed a BZEST for application in the shrimp farming sector, which is characterized by the improved productivity and better FCR. This BZEST system is amenable for control of disease through Best Management Practices and preservation of water resources.

II. Species Diversification

CIBA has successfully bred several candidate species of brackishwater finfish and shellfish. The hatchery production technology of several of the diversified finfish species such as Asian sea bass (*Lates calcarifer*), grey mullets (*Mugil cephalus*), milkfish (*Chanos chanos*), pearl spot (*Etroplus suratensis*) are available for brackishwater aquaculture. Nursery rearing and grow-out technologies for few of the candidate species have been developed and demonstrated. Similarly, mud crabs, species of *Scylla*, are one of the most traded sea food commodities in India. The technology for seed production, nursery rearing and grow out production have been standardized and under various stages of technology transfer.

Other Penaeid species: Species such as *Penaeus indicus F. merguinesis, Marsupenaeus japonicus* and species of genus *Metapenaeus*, are valuable species for aquaculture in India. The hatchery production of these species are standardized and many experimental grow out culture have been conducted. These species have regional importance and have high market values. Strategies have to be developed for the popularization of these species.

Indigenous vis-à-vis exotic species

Availability of captive broodstock; ability to conduct domestication and genetic selection work; availability of SPF and SPR lines; elimination of problems associated with wild broodstock and/or PL collection; and faster generation times are the advantageous attributed to *P. vannamei* growth. However, few of the indigenous species holds excellent potential for growth and can be adopted for profitable coastal aquaculture.

Penaeus indicus: The Indian white shrimp

The Indian white shrimp, *Penaeus indicus*, a native candidate shrimp of Indian subcontinent, and cultured in India, Middle East and Eastern Africa is a proven brackishwater aquaculture species, one of the first shrimp species farmed along the coastal region till 1990s, prior to the demonstration of scientific shrimp farming using tiger shrimp. Commercial shrimp farming with selective stocking of shrimp seed was initiated with the establishment of shrimp hatcheries of *P indicus* and *P. monodon* in India in the late 1980s. However, due to predominance of commercial tiger shrimp culture from 1990's due to farmer's preference and better economic returns over investment, Indian white shrimp has not been given the prominence that it deserves. Subsequent to the outbreak of WSSV in Asia and India since

1994, and related crop failures among the Indian shrimp farming, genetically improved exotic specific pathogen free (SPF) shrimp, the *Penaeus vannamei*, got introduced to India to combat the WSSV inflicted disease problems. The introduction of vannamei now register a total take over in the shrimp farming scenario, registering a farming coverage of almost 90%. Of the 0.45 million tonnes of farmed shrimp produced in India during 2014-15, almost 0.37 million tonnes is vannamei. This total dependence of shrimp farming on a single exotic species, with emerging problems of inbreeding, issues in seed quality and emerging disease problems necessitates the need of taking up a proven candidate species native to Indian subcontinent like indicus, for stock improvement through a selective breeding programme. As a nodal institute of brackishwater aquaculture R&D in India, ICAR-CIBA has evaluated the status of scientific data already available with species, with special reference to a stock improvement programme. Basic data set such as information on the genetic stock structure, and stock variation was not available on *P. indicus,* which is required for initiating the selective breeding programme in this species.

There is a huge potential for the standardization of Indian white shrimp, endemic to the Indian coast for upgradation of breeding and seed production through stock evaluation program. In this backdrop, a culture demonstration program has been initiated under the funding of NFDB to investigate the performance of Indigenous Indian White shrimp, with components of stock evaluation, hatchery production of seed and scientific farming. Different stocks across Indian coast are compared for their culture potential to mark the base line for the genetic improvement program and to evaluate the merit of promoting our own indigenous species highlighting “Make in India” *vis-à-vis* exotic species. This effort will provide first line information required to start a selective breeding programme for the desi shrimp *P. indicus,* as an alternative to American shrimp vannamei.

ICAR-CIBA has taken up demonstration of high semi-intensive culture practices of this shrimp over a range of production systems across different states to increase adoption by farmers. Captive breeding of Indian white shrimp is refined and production of high quality disease free seeds is possible to be produced. The seed production technique was refined, and subsequent farming demonstration evinced interest in farmers to culture Indian white shrimp, thus making Indian *P. indicus* a potential species for diversification. Multi-location culture trial of Indian white shrimp *P. indicus* was shown to be successful and reveals following aspects.

Species suitability for high density culture:

- It has excellent growth potential and up to 18-20 g grows better or as fast as L.vannamei across different culture system
- It is easier to culture in high stocking densities

  Initial culture studies have shown that a production of 3-4 tons/ha can be achieved at 25-30 nos/sqm nearly at par with P. vannamei at similar stocking density. Further demonstration trials aiming higher level of production are underway in multi-location across India.

  With further intensification, a production of 5-7 tons is reported to be achieved in this species. A density dependent growth pattern was observed.
- It is tolerant to wide range of salinities 5 to 45 ppt (except very low to zero salinity)
- This species is found to be compatible in the polyculture system with other fin fishes like mullets, milkfish and pearl spot and with other Penaeid shrimps.
- Various eco-based culture techniques and their potential for high density culture and technical efficiency of this species are being evaluated and initial studies are encouraging.
- It is an easy to breed this species and hence domestication of the species is very much possible with the production of SPF stock because of the shorter generation period and easier captive breeding.
- Has similar market values like other white shrimps like *L. vannamei*

P. indicus as Demonstration trials were conducted to evaluate the performance of Indian White Shrimp *Penaeus indicus* under NFDB sponsored project titled "Upgradation of Breeding and Culture Technology of Indian White Shrimp *Penaeus indicus* through stock evaluation and culture Demonstration"**.** Under the program, the different stocks, viz., East coast (Odisha), South East Coast (Tamil Nadu) and South West Coast (Kerala) are procured, transport and quarantine to ensure the utilization of healthy broodstock for breeding purposes. The seeds produced from different stocks are evaluated through culture demonstration. Presently, the culture demonstration is carried out under monoculture/polyculture mode in six locations of different coastal states like Andhra Pradesh, Kerala, Gujarat, West Bengal, Odisha and Tamilnadu.

The grow-out culture demonstration of *Penaeus indicus* was carried out in low salinity water at Kakdwip from July to Nov, 2016. Quality disease free seeds produced at

CIBA and other hatcheries near Chennai (TN) were stocked at a density of 25 -35 pc/ sq m and reared for four months based on biosecured zero water exchange systems. Indian white shrimps reared with indigenously developed feed reached an Average Body Weight between 18-20 grams and a production of up to 3.-5 tonnes per ha was achieved. A higher survival up to 75 % was achieved in spite of extremely low salinity during the culture. Indian white shrimp registered better or comparable growth to that of pacific white shrimp *L. vannamei* up to 16-17g. Salinity during the culture period ranges from 2-12 ppt. In another experiment at Balasre, Odisha, at the end of 125 -130 days of culture period, a final body weight of 28-30g was recorded at lower stocking density (10pc/sq m) whereas at higher stocking density (35 pc/ sqm), final body weight of 17-20 g was recorded, respectively. A production of 3 to 6 tonnes per ha is possible in this shrimp.

Penaeus merguiensis: The Banana shrimp

Almost similar to *P.indicus* occuring along the Indian Ocean, Oman to Indonesia and Australia. It can be grown at high densities and resistant to lower temperatures up to $15^{O}C$ with quick turnover rate in ponds of sizes of 10-20 g at high density and 20-30 g at low density can be attained after three months with harvests of 300-400 kg/ha/crop. Moreover, an ideal alternate crop to *P. monodon* during dry months.

Thus, the main reasons for diversification of species is a necessity to resolve some of the compounding issues such as

- Countering some of the diseases that are rampant for now dominating in cultured species which may not be as potent in the new introduced species
- Highly dynamic international prices can be controlled with the shortage of dominating species at a time
- Improvement of domestic market with replacement of short duration small size culture able species
- Development of localized and native species culture for domestic and international market based on demand

4. Genetic Improvement program and SPF development

The native species of each country has its potential for diversification. The Genetic improvement program of Indian White shrimp will aim to sustain the Indian Shrimp Aquaculture industry through the supply of domesticated SPF (Specific Pathogen Free) Indian White Shrimp broodstock for seed production and farming thereby augmenting aquaculture production and export. There is enough indication through preliminary trials that this species could be an indigenous alternative for *P. vannamei*. Commercial availability of SPF or High-health stock will be an added advantage.

P. indicus Research and high-density culture demonstration by ICAR-CIBA

5. Conclusion:

India has vast resources of Inland saline water which are close to nature and can be easily modified to suite these technologies. Cost effective strategy are required for this area to cope with the elevated energy and major nutrients requirement for immune augmentation, maintenance of homeostasis and growth with targeted use of dietary additives and balanced nutrients for adaptation of shrimp and fish in inland saline aquaculture. Most innovations and development of brackishwater aquaculture show-casing the economic earnings is mainly due to the "industrial" aquaculture, using SPF seeds, formulated extruded feeds, aeration and with the use of various pond management inputs. ICAR institutes like CIFE and CIBA have developed and demonstrated some of the farming-based technologies in these eco-systems; and dissemination of other eco-based technology like organic farming system; including periphyton based farming, polyculture, biofloc and biomimicry-based technology

Bibliography

1. Allan, Geoff L., D. Stewart Fielder, Kevin M. Fitzsimmons, Samuel L. Applebaum, and Sudhir Raizada. "Inland saline aquaculture." In *New technologies in aquaculture*, pp. 1119-1147. Woodhead Publishing, 2009.
2. Ansal, Meera D., and Prabjeet Singh. "Development of inland saline-water aquaculture in Punjab, India." *Global Aquaculture Advocate. https://www. aquaculturealliance.org/advocate/development-inland-saline-water-aquaculturepunjab-india/? headlessPrint= AAAAAPIA9c8r7gs82oW* (2019).
3. Aklakur, M. D. "Nutritional intervention for sustainable production in inland saline aquaculture a budding perspective in India." *Journal of Aquactic Marine Biology* 6 (2017): 172.
4. Boyd, C.E., Thunjai, T. and Boonyaratpalin, M., 2002. Dissolved salts in water for inland low-salinity shrimp culture. Global Aquaculture Advocate, 5(3), 40-45.
5. Avnimelech Y. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture. 1999 Jun 15;176(3-4):227-35.
6. Khanjani MH, Mohammadi A, Emerenciano MG. Microorganisms in biofloc aquaculture system. Aquaculture Reports. 2022 Oct 1;26:101300.
7. de Souza DM, Suita SM, Romano LA, Wasielesky Jr W, Ballester EL. Use of molasses as a carbon source during the nursery rearing of Farfantepenaeus brasiliensis (Latreille, 1817) in a Biofloc technology system. Aquaculture Research. 2014 Jan;45(2):270-7.
8. Ekasari J, Suprayudi MA, Wiyoto W, Hazanah RF, Lenggara GS, Sulistiani R, Alkahfi M, Zairin Jr M. Biofloc technology application in African catfish fingerling production: the effects on the reproductive performance of broodstock and the quality of eggs and larvae. Aquaculture. 2016 Nov 1;464:349-56.
9. Emerenciano M, Martinez-Córdova LR., Martínez-Porchas M, Miranda-Baeza A (2017) Biofloc technology (BFT): a tool for water quality management in aquaculture. In: Water quality. Tutu H. (ed), IntechOpen
10. Miao S, Hu J, Wan W, Han B, Zhou Y, Xin Z, Sun L (2020) Biofloc technology with addition of different carbon sources altered the antibacterial and antioxidant response in *Macrobrachium rosenbergii* to acute stress. Aquaculture. 25:735280.
11. Nicholson JK, Holmes E, Wilson ID. Gut microorganisms, mammalian metabolism and personalized health care. Nature Reviews Microbiology. 2005 May;3(5):431-8.

12. Panigrahi A, Esakkiraj P, Das RR, Saranya C, Vinay TN, Otta SK, Shekhar MS. Bioaugmentation of biofloc system with enzymatic bacterial strains for high health and production performance of Penaeus indicus. Scientific reports. 2021 Jul 1;11(1):1-3.
13. Panigrahi A, Esakkiraj P, Jayashree S, Saranya C, Das RR, Sundaram M. Colonization of enzymatic bacterial flora in biofloc grown shrimp Penaeus vannamei and evaluation of their beneficial effect. Aquaculture International. 2019 Dec;27(6):1835-46.
14. Panigrahi A, Sundaram M, Saranya C, Swain S, Dash RR, Dayal JS (2019) Carbohydrate sources deferentially influence growth performances, microbial dynamics and immunomodulation in Pacific white shrimp (*Litopenaeus vannamei*) under biofloc system. Fish shellfish Immunol 86:1207-16.
15. Zhao P, Huang J, Wang XH, Song XL, Yang CH, Zhang XG, Wang GC (2012) The application of bioflocs technology in high-intensive, zero exchange farming systems of *Marsupenaeus japonicus*. Aquaculture 354:97-106.

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Post-harvest processes in fish/shrimp farming to retain the nutritional value of fish/shrimp

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Fish are considered a nutritionally valuable part of the human diet and consumption two times a week is recommended, mostly due to the content of long-chain polyunsaturated n-3 fatty acids. These fatty acids are essential in human nutrition and have proven to be involved in many metabolic functions. Among others, they have anti-inflammatory effects, decrease platelet aggregation and are essential parts of the cell membranes, cardiovascular system, brain, and nervous tissue. In addition, the proteins, peptides and amino acids from fish became more recently known for having positive health effects. Furthermore fish is also a rich source of certain vitamins and minerals such as Vitamin D, selenium, phosphorus, and calcium.

India's domestic market for shrimp consumption remains less than 50,000 MT per year. Stimulation of this market could lead to a significant increase in fish consumption. Despite high fish production, fish consumption in the country remains low, so to be increased. The rising urban middle class, the large population of young people, the convenience of cooking shrimp/fish and the health benefits of seafood are being leveraged to create a high-value domestic market for shrimp in India. The average annual per capita fish consumption is highest in Kerala state at 30 kg/person and Tripura at 25 kg followed by Lakshadweep, Goa, Kerala, Arunachal Pradesh, and Andaman and Nicobar Islands. In terms of per capita fish consumption, Korea scored highest (78.5 kg), Norway (66.6 kg), Portugal (61.5 kg), Myanmar (59.9 kg), Malaysia (58.6 kg) and Japan (58 kg) – China comes in seventh at 48.3 kg per capita.

The per capita consumption of fish in Tamil Nadu is 9.83 Kg as against the recommended requirement of 11.60 kg. With the increase in demand for fish, there is a need for augmenting the overall fish production of the State substantially from all the available fishery resources. However, aquaculture is emerging as a prominent activity for enhancing fish production and income generation in rural areas. Integration of fish culture with agriculture has proved to be an option for augmenting the unit productivity of aquaculture systems

It estimates that around one-third of the world's fish stocks are overexploited, and populations are declining because we're catching more fish than can reproduce. The average per capita fish consumption in India is 9 kilograms (kg) /per year against the global per capita fish consumption of 16 kg. The World Health Organization (WHO) recommends consuming 12 kg of fish in a year. Processed fish products exhibit numerous potential health benefits like stability as compared with conventional foods, due to controlling deteriorative reactions during storage. Processed foods fulfil the food desires of the consumers and seafood choice is influenced by various factors, biological processes, psychological conditions, sociocultural relations, economic values, and environmental factors.

Shrimp farming is an aquaculture business that exists in either a marine, brackish or freshwater environment, producing shrimp or prawns for human consumption. India is the world's second-largest producer of white-leg shrimp (13% of global production) and 98 per cent of farmed shrimp produced in India. Additionally, this sustainable approach to shrimp farming means that shrimp prices will increase and so will the incomes of these farmers, providing shrimp farmers with an incentive to practice sustainable shrimp farming and addressing the growing demand for shrimp while also conserving mangroves.

Harvest of shrimps from the grow-out ponds:

1. The condition of shrimps in the grow-out pond has to be estimated before harvest for moulting- harvest shall not be exercised during moulting as it going to gain weight in a few days. Hence the farmer has to give supplementary feeding to become shell hardening and then harvesting will be ideal
2. The farmer can use cast net /check tray to test moulted shrimps from the pond before harvest.
3. The process of harvest should be initiated during the early hours or late evening, accordingly, 30 cm of water level may be reduced from the grow-out ponds and a big harvest bag made of nylon is to be tied in the outlet of the pond
4. The shrimps shall not be allowed inside the grow-out ponds for a long time as their rapid movement will get tired and which may reflect or enhance more rigor mortis stage that leads to more spoilage
5. The shrimps shall not be allowed in the very low water level for a long time as low water may develop stress to the shrimps

6. The harvested shrimps from the grow-out ponds may be segregated for unwanted fish/Crabs and then subjected to chill killing
7. Chill killing is the process of subjecting harvested shrimps to ice water (4^0C) for 5 min, this step will rapidly kill the shrimps and the bacterial spoilage will be minimised
8. The chill-killed shrimps have to be packed in plastic crates with a 1:1 ration of powdered ice with a minute hole in the crate to expel the water. The powdered ice will have uniform cooling to the shrimps or fish
9. The packed shrimps are to be taken to the shrimp processing plants for processing through the insulated vehicle
10. The shrimps have to be washed in 10ppm chlorinated water and then graded as per the buyer's specifications and then packed in a wax-coated duplex cartoon
11. The packed shrimps in the duplex carton are to be frozen in the plate freezer at -40^0C/90 min

Icing is the most prevalent method of preserving fish. Ideal icing involves packing crushed ice and fish alternatively in insulated boxes, in the fish-to-ice ratio of 1:1 (w/w). By this, the temperature of the fish is lowered to nearly 1 to 2°C in about 2-3 hrs (the melting of the ice needs 80 calories of heat/g and this heat is removed from the fish in contact with ice and hence, the fish get cooled). Lowering temperature brings about arresting all enzymatic changes, killing about 50-60% of the mesophilic bacteria and slowing down the activities and growth of all other bacteria, which are cold-loving (psychrophilic) and cold-tolerant (psychrotrophic).

As a combined effect of all these three factors, the spoilage of fish is delayed to a considerable length of time in ice. During iced storage of fish, there is an initial drop in bacterial count due to the death of the cold-sensitive mesophiles. The surviving cold-tolerant bacteria, however, get adapted to growth in low temperatures. Consequently, there is a gradual increase in population, which takes about 6 to 8 days to reach a count of one million per gram or above. By that time, the fish has reached the stage of incipient spoilage. Qualitatively, there is a selection of bacterial flora during the iced storage of fish. Irrespective of the composition of the initial flora, the *Pseudomonas*/ *Alteromonas* group emerges as the predominant group of bacteria at the time of spoilage.

Advantages of chilling fish with ice soon after harvest

i) ***Temperature reduction.*** It reduces the temperature to about 0^oC. The growth of spoilage and pathogenic microorganisms is reduced, thus reducing the spoilage rate.

ii) ***Melting ice keeps fish moist.*** This action mainly prevents surface dehydration and reduces weight loss. Melting water also increases the heat transport between fish and ice surfaces

iii) ***Advantageous physical properties.*** Ice has some advantages when compared with other cooling methods, including refrigeration by air. The properties can be listed as follows:

a) ***Ice has a large cooling capacity.*** *The latent heat of the fusion of ice is about 80 Kcal/kg. This means that a comparatively small amount of ice will be needed to cool 1 kg of fish.*

b) ***Ice melting is a self-contained temperature control system.*** Ice melting is a change in the physical state of ice (from solid to liquid), and it occurs at a constant temperature (0^oC).

The overall reason for icing fish is to extend the shelf life fresh fish of in a relatively simple way as compared to the storage of un- iced fish at ambient temperatures above 0^oC. Extension of shelf life means producing safe fresh fish of acceptable quality.

Handling shrimp on shore

Whole raw shrimp should be processed at factories close to the ports of landing. Unless freezing facilities are available on board the catcher, all processing, including cooking is better done ashore. Inshore species of shrimp as well as deep water shrimp can be cooked and further processed on shore under more hygienic conditions with little loss of quality if they are iced at sea immediately after capture.

Freezing of whole shrimp

The methods described earlier for freezing shrimp at sea are equally applicable on shore, provided the chilled raw material is frozen within 2-3 days of capture; the thawed product can then be used for further processing in the same way as fresh shrimp.

Cold storage of whole shrimp

Whole shrimp, raw or cooked, frozen individually in air blast or blocks with water in a plate freezer, will keep in good condition in a cold store at -30°C for at least 6 months. Individually frozen whole shrimp will keep for 3-4 months in good condition at -20°C, and for only 1 month at -10°C; whole shrimp stored at -10°C are more difficult to peel when thawed. Blocks of shrimp with added water will keep a little longer at these temperatures, up to 6 months at -20°C and 2-3 months at -10°C. It is recommended that wherever possible whole shrimp being stored for an indefinite period should be kept at -30°C. Raw and cooked frozen whole shrimp develop cold store odours and flavours during storage, and the higher the storage temperature, the more quickly they develop. Shrimp cooked after freezing and cold storage are usually paler in colour than shrimp that are cooked before freezing. Both raw and cooked whole shrimp must be adequately protected against dehydration during cold storage, either by glazing or by suitable packaging; the shell of the shrimp provides no protection. Glazing should be inspected periodically and renewed as required.

Thawing of frozen whole shrimp

Blocks of whole shrimp frozen at sea or on shore can be thawed in the air or water. Thawing times for a typical commercial block measuring 1050 mm × 530 mm × 50 mm thick and containing about 18 kg shrimp and 6 kg water are as follows; 20 hours in still air at 18°C, 2 hours in saturated moving air at 18°C, 1½ hours immersed in water at 18°C and 1 hour in a water spray at 18°C. The water spray method is the fastest because the fine jets help to break up the block as individual shrimp thaw on the surface, thus exposing a greater surface area. With each of the methods, the blocks are soft enough to break up by hand before the shrimp are fully thawed, but it is difficult to do this without damaging some of the shrimp.

The thawed shrimp can be further processed in the same way as whole chilled shrimp. Individually frozen shrimp can be thawed in a few minutes, or they can be cooked directly from the **frozen state.**

Size grading

Whole raw shrimp on receipt at the factory are first graded for size, since large shrimp are generally more valuable than small ones, and mechanical peelers require a supply of shrimp within a fixed size range. Small shrimp that are uneconomic to peel are either discarded or used in the chopped form as raw material for various products.

Cooking

Shrimp are cooked to provide a product that is ready to eat, and to loosen the meat in the shell before peeling.

The cooking process can be more easily controlled on shore than at sea; more space is available, better heating systems can be used, and instrumentation is more practicable. A short brisk cook is better than a long slow one. The ratio of shrimp to water should be as low as possible so that the water returns to a boil as quickly as possible after the shrimp have been put in. With a ratio of 1 kg shrimp to 20 litres of water, the temperature of the water will fall initially to about 95°C, and there should be sufficient heat available to bring it back to a boil in 1-2 minutes. The water in the boiler should contain 3-5 per cent salt; the use of stronger brines can cause discolouration of the meats during subsequent chilled storage.

Cooking time is important; about 3 minutes is usually sufficient for UK shrimp, but the precise time for a certain size or quality of shrimp should be determined by experiment. The boiling time should be long enough to develop fully the flavour and texture of the shrimp meat, and to loosen the meat from the shell; overcooking can destroy the flavour and can cause loss of weight. A lidded wire mesh basket can be used for immersing the batches of shrimp, and the basket of shrimp should be agitated gently in the water to ensure uniform cooking. Scum should be removed from the surface of the water as often as possible, and the water in the boiler changed frequently, preferably several times a day.

Cooling

The shrimp should be cooled immediately after cooking. Cooling in the air is claimed to give the whole cooked shrimp a better colour but, unless the shrimp are to be marketed in this form, it is recommended they be cooled in water. The yield of meat from water-cooled shrimp can be up to 4 per cent higher than from air-cooled shrimp. They can either be immersed directly into chilled water for about 3 minutes until they are at a temperature of about 0°C, or they can be cooled in two stages, first in water at tap delivery temperature and then in chilled water. The latter method will be more economic in terms of ice or mechanical refrigeration. The shrimp should never be left to soak any longer than is necessary to chill them. As soon as they are down to chill temperature, they should be lifted out, drained, packed in clean boxes and transferred either directly to the peeling area or into a chill room. Water and ice used for cooling must be clean; hygiene is important, and a chlorination system may be necessary.

Peeling

Brown shrimp and pink shrimp from inshore waters are still normally peeled by hand, but machines are now available that will handle the larger deep water shrimp. In hand peeling, the body of the shrimp is held in one hand, and the head twisted off with the other. The first two or three segments of the shell are then broken open with the thumb, and the tail is squeezed to release the meat. A skilled worker can peel 2½-3 kg whole shrimp an hour. Shrimp are often consumed without any further cooking; therefore particular attention must be paid to hygiene and sanitation. Regular bacteriological control of processing is important for this type of product. The main source of bacterial contamination of cooked shrimp is the peeling process, particularly hand peeling. Workers must wash their hands frequently, and all working surfaces must be kept scrupulously clean. Recommended procedures for factory cleaning are given in Advisory Note 45 ‘Cleaning in the fish industry. The use of chlorinated water on the processing line can be of considerable help in keeping down contamination, but shrimp waste must not be allowed to accumulate since the chlorine is rapidly inactivated by protein. The shrimp must be kept cool throughout the process.

Machine peeling results in far less recontamination of cooked shrimp than hand peeling. Provided the peeling machines are cleaned at frequent intervals, the risk of increasing spoilage or introducing food poisoning bacteria is much less with machine peeling than with hand peeling. Where raw meats are required for further processing, thawed frozen whole shrimp are much easier to peel than very fresh unfrozen shrimp. Peeling of unfrozen shrimp becomes easier after 1-2 days of chilled storage.

Yield and packing density

The yield of meat from whole shrimp is variously quoted as ranging from 20 to 45 per cent, but it is not always specified whether the yield is from raw or cooked whole shrimp. The head constitutes about 40 per cent of the weight of whole raw shrimp, and the tail shell and legs a further 15 per cent; the yield of raw meat is thus about 45 per cent. The yield of cooked, peeled meats from samples of raw whole deep water shrimp, weighed first after freezing and thawing and again after cooking and hand peeling, is about 28 per cent. The weight loss during a 3-minute cook is about 26 per cent, and a further 46 per cent is lost during hand peeling. If the initial weight is taken to be that of the whole cooked shrimp, the yield on hand peeling is about 38 per cent.

Dipping

Several dipping treatments between peeling and freezing of deep-water shrimp have been tried in British trade practice, to improve flavour or colour. For example, salt, monosodium glutamate, citric acid or sodium citrate, polyphosphate and dye have all been, or are being, used at some stage in the process. The value of some of these treatments is questionable. Where dipping solutions are used, they should be kept chilled and renewed at frequent intervals to prevent bacterial contamination of the cooked meats. The meats are sometimes dipped in a 3-7 per cent salt solution when the salt uptake during boiling is found to be insufficient. Monosodium glutamate is used to enhance flavour, and the use of polyphosphate is claimed to reduce weight loss on thawing. Citric acid or sodium citrate is used to reduce the discolouration of meats, and dye is used to give a uniform pink colour; the dye is sometimes added at the cooking stage. Most if not all of these treatments should be unnecessary on good quality shrimp meats handled and processed expeditiously.

Freezing of meats

The peeled meats can be frozen individually or in blocks. Individually quick frozen, IQF, meats are particularly suitable for catering and retail outlets since the required amount can be dispensed from the pack without thawing. Meats in blocks suffer less weight loss during freezing, and are better protected in cold storage, but have the disadvantage that a complete block has to be thawed and used at one time. IQF meats can be frozen in either an air-blast freezer or a liquid nitrogen freezer. The air blast freezer can be either a batch or continuous type, but delays between the freezer and cold store are more likely with a batch freezer, and individual small meats can warm quickly during this time; continuous freezers are therefore recommended to ensure a steady flow of frozen meats from freezer to the cold store. The fluidised bed type of freezer is used for freezing individual shrimp but, where the meats are moved through the freezer entirely by agitation in air, there is some weight loss in the form of pulped meat or mush, which may have to be recovered and utilized in some way. A modified form of fluidised bed freezer is available that overcomes this difficulty by agitating the meats just enough at the start of freezing to ensure that they are individually frozen and then moving them by belt through the remainder of the freezing process. IQF meats of deep water shrimp require a freezing time of about 10 minutes at -30°C in air moving at 5 m/second. Liquid nitrogen freezers are compact and can freeze shrimp meats quickly; a freezing time of 3½-5 minutes is typical, but they are expensive to operate, and a high degree of utilization is essential to keep down the cost. Sheets of plastic film or trays with non-stick surfaces may have to be used to prevent the meats from adhering to the freezer belt. If the meats are not laid out individually, some inevitably stick together, and the separation of these before packing adds to the labour cost. Blocks of shrimp meats are normally prepared by packing the meats into trays or moulds and freezing them in a horizontal plate freezer. The trays are slightly overfilled so that there is compacting during freezing to give a homogeneous block. A typical block is 25-30 mm thick. The freezing time for a 25 mm block in a horizontal plate freezer operating at -35°C is about 40 minutes. The frozen blocks are usually packed in cartons with an inner wrapper, and then moved to a cold store. Alternatively, the shrimp may be cartoned before freezing; the freezing time for a 25 mm block in a carton is about 50 minutes.

Packing and glazing

Individually quick frozen meats for sale to caterers and retailers are normally weighed into flexible film bags which are sealed and packed in fibreboard outer cartons for storage and distribution. The film used for the individual packs should have a high resistance to the passage of water vapour and oxygen so that dehydration and oxidation are kept to a minimum; for example, a laminate of polyethene and polyester, or a single polyamide film, is suitable. The individual meats are often glazed, that is dipped in cold water to coat them with ice, before packing them, to protect the product against drying in cold storage. However, packing in sealed film bags is sufficient to protect the unglazed product under good commercial conditions. Vacuum packing will give added protection against the possibility of occasional poor commercial cold storage. It is extremely difficult to control the amount of ice picked up by small meats during glazing, due to variations in the size and temperature of the frozen product, the temperature of the glazing water, and the duration of the dipping or spraying process. The proportion of glaze to total glazed weight can vary from 10 to 40 per cent. The weight of glaze is usually included in the declared weight of contents of consumer packs, and the resulting loss of weight on thawing can cause customer dissatisfaction. Successful prosecutions have been brought against processors for excessive application of glaze to shellfish meats. The addition of glaze also results in considerable warming of the product, and it may be necessary to refreeze to avoid imposing an excessive heat load on the cold store. Meats frozen in liquid nitrogen are often at a low enough temperature to cause the glaze to shatter and become ineffective as a protective barrier. For all these reasons it is strongly recommended that the practice of glazing individual meats for sale to caterers and retailers be discontinued and replaced by adequate packing.

Blocks of shrimp meats can be glazed as an alternative to protective packaging, or they can be wrapped in a suitable plastic film and packed in fibreboard outers. Bulk lots of individually frozen meats awaiting further processing can also be glazed before storage. It must be remembered that glazed products in cold stores must be inspected periodically and the glaze renewed as required.

Cold storage of meats

Frozen cooked shrimp meats should be stored at 30°C, they will keep in good condition at this temperature, provided they are properly wrapped or glazed, for at least 6 months. Long-term storage at higher temperatures is not recommended; for example, after 3-4 months at -20°C, the development of undesirable odour and flavour and poor texture can make the product unacceptable.

Other shrimp products

Shrimp meats can be used in the preparation of several seafood products, including pastes, spreads, crisps, soups, bisques, sauces and other prepared dishes, most of which can be stored for long periods after freezing or canning. It is not possible within the space of this note to give individual methods of preparation.

Composition of shrimp: Raw shrimp meat contains 75-80 per cent water, 18-20 per cent protein and about 1 per cent fat; cooked meat contains 65-70 per cent water, 25-30 per cent protein and about 1 per cent fat. The calorific value of cooked shrimp meat is about 4.5 kJ/g. Vitamins A and D are present in small quantities.

Various other processing methods used for fish/shrimps:

1. *Icing*
2. *Salting*
3. *Freezing*
4. *Canning*
5. *Curing/Drying*
6. *Smoking*
7. *Grilling*
8. *Shallow/deep fat frying*
9. *Value addition*
10. *Blanching*

11. *By-products preparation*
12. *Broiling*
13. *Pickling*

In India, the overall per capita fish consumption is lower than the other countries and WHO is recommending consuming 12 kg of fish per person /annum. Awareness should be created to maintain the cold chain among the fish sellers and consumers as fish/shrimp is a highly perishable commodity as 70 % of the total fat is unsaturated. Much more research has to be carried out to mask the fresh fish odour from the selling markets as this may diminish the preference for fish purchase and also more value-added fish products outlets to be opened up as compared to dairy products in India

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Diseases and health management in *Penaeus vannamei* shrimp farming

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1. Introduction

Shrimp farming is one of the promising enterprises in the current scenario providing employment opportunities to many. This sector is gaining impetus day by day and many are involved in shrimp aquaculture. As the intensification of shrimp aquaculture begins there are also so many adversities it has to encounter. Of which the major one is diseases, and it does not give any time for the farmer to think and thereby it is causing loss to the farmer. In this article, brief outlines on important shrimp diseases which can have implications on shrimp aquaculture are discussed.

2. Viral Diseases

2.1 White spot disease (WSD)

This virus is the most serious threat facing the shrimp farming industry. WSSV was first reported in farmed *P. japonicus* from Japan in 1992/93, and was thought to have been imported with live infected PL from Mainland China. WSD is caused by one of the largest animal viruses. WSSV is a large ds DNA virus of 120-150 x 270-290 nm size, assigned to a new virus family, *Whispoviridae*. WSSV can infect a wide range of aquatic crustaceans including marine, brackish and freshwater penaeids, crabs and crayfish. All penaeid shrimp species are highly susceptible to infection, often resulting in high mortality. Crabs, crayfish, freshwater prawns, spiny lobsters and clawed lobsters are susceptible to infection, but morbidity and mortality consequence of infection is highly variable. Prevalence of WSSV is reported highly variable, from <1% in infected wild populations to up to 100% in captive populations. WSSV affects most organs derived from ectodermal and mesodermal origin, including the cuticular epithelium, connective tissue, nervous tissues, muscle, lymphoid organ and haematopoietic tissues. The virus also severely damages the stomach, gills, antennal gland, heart and eyes. During later stages of infection, these organs are destroyed and many cells are lysed. The shrimp then show reddish discolouration of the hepatopancreas and characteristic 1-2 mm diameter white spots on their carapace, appendages and inside surfaces of the body. Affected shrimp show lethargic behaviour. Cumulative mortality typically reaches 100 percent within two to seven days of infection. The mode of transmission of WSD around Asia was believed to be through exports of live PL and broodstock. The infection can be transmitted vertically, horizontally by cannibalism, predation, etc. and by water-borne routes. Dead and moribund animals can be a source of disease transmission. However, some studies have shown that disinfection of water supplies and the washing and/or disinfection of the eggs and nauplius is reported to be successful in preventing its transmission from positive broodstock to their larvae. It is generally believed that the virus sticks to the outside of the egg, and if it gains entry to the egg, it is rendered infertile and will not hatch. Thus, using proper testing and disinfection protocols, vertical transmission can be prevented in the hatchery. It has now become clear that the presence of WSSV in a pond does not always lead to disaster.

Shrimp infected with WSSV displaying white spots on carapace

The disease can be visually diagnosed through the presence of the characteristic white spots, which can be seen in advanced stage of infection. However, white spots may not be always present in infected shrimp. WSSV can be detected by using PCR, or with probes for dot-blot and *in situ* hybridization tests. PCR detection efficiency can be increased by exposure to stressful conditions (e.g. eye-stalk ablation, spawning, moulting, changes in salinity, temperature or pH, and during plankton blooms). WSSV can be confirmed histologically (particularly in asymptomatic carriers) by the presence of large numbers of Cowdry A-type nuclear inclusions and hypertrophied nuclei in H&E-stained sectioned tissues, or simply by rapid fixation and staining of gill tissue. At present there is no treatment available to prevent the unrestrained occurrence and spread of the disease. Broodstock should be PCR screened before breeding. PL should also be PCR screened before stocking into ponds, as this has been proven to result in a higher percentage of good harvests. Washing and disinfection of eggs and nauplii has also been shown to prevent vertical transmission of WSSV from infected broodstock to larval stages. The white spot virus only remains viable in water. Hence, all effluent from farms or processing plants should be disinfected with formalin or chlorine prior to discharge. Better management practices (BMPs) have helped alleviate this problem to a great extent, by minimizing risks of its transmission through carrier organisms such as mud crabs, Artemia, rotifer eggs, molluscs, polychaete worms, insect larvae and seabirds etc.

2.2 *Infectious Hypodermal and Haematopoietic Necrosis (IHHN)*

Infectious Hypodermal and Haematopoietic Necrosis was first reported in *P. stylirostris* from America in the year 1981. It was thought to have been introduced along with live *P. monodon* from Asia. Recent studies have revealed geographic variations in IHHNV isolates, and suggested that the Philippines was the source of the original infection in Hawaii, and subsequently in most shrimp farming areas of Latin America. Large-scale epizootics were responsible for multi-million dollar losses in *P. vannamei* culture in the Americas during the 1990s. IHHNV is caused by a small (20-22 nm) single-stranded DNA Brevidenso virus. Gross signs of disease are not specific to IHHN, but may include reduced feeding, elevated morbidity and mortality rates, fouling by epicommensals and bluish body coloration. Larvae, PL and broodstock rarely show symptoms. In *P.vannamei*, IHHNV can cause runt deformity syndrome (RDS), which typically results in cuticular deformities (particularly bent rostrums), slow growth, poor FCR and a greater size variation at harvest, contributing substantially to reduction in profits.

Shrimp infected with IHHNV displaying size variation and slow growth

IHHNV typically causes no problems for *P. monodon* since they have developed a tolerance to it over a long period of time, but they may suffer with RDS. *P.merguiensis* and *P. indicus* appear refractory to the IHHNV. However, these species may be life-long carriers of the virus and could transmit the virus to *P. vannamei*, which typically suffer from RDS due to IHHNV infection. IHHNV can be diagnosed using methods such as DNA probes in dot blot and *in situ* hybridization and PCR techniques (including real-time PCR) as well as histological analysis of H&E-stained sections looking for intracellular, Cowdry type A inclusion bodies in ectodermal and mesodermal tissues such as cuticular epithelium, gills, foregut, hind gut, lymphoid organ and connective tissues. Transmission of IHHN is known to occur rapidly by cannibalism shrimp. It can also be transmitted through waterborne route and cohabitation. Vertical transmission from broodstock to larvae is common. Strict hatchery biosecurity including checking of broodstock by PCR, or the use of SPF broodstock, washing and disinfecting of eggs and nauplii is essential in combating this disease.

2.3 Infectious Myonecrosis (IMN)

Infectious myonecrosis is an emerging *P. vannamei* disease, first detected in Brazil during 2004, and then in Indonesia in 2006. IMN has been detected in Indonesia and Brazil. The principal host species is *P. vannamei* in which IMNV known to cause significant disease outbreaks and mortalities. IMN is caused by putative Totivirus. IMNV particles are icosahedral in shape and 40 nm in diameter. Juveniles and sub-adults of *P. vannamei*, farmed in marine or low salinity brackish water, appear to be the most severely affected by IMN disease. The principal target tissues for IMNV include the striated muscles (skeletal and less often cardiac), connective tissues, haemocytes, and the lymphoid organ parenchymal cells. IMN disease causes significant mortality in grow-out ponds and is characterized by acute onset of gross signs including focal to extensive whitish necrotic areas in the striated muscle, especially of the distal abdominal segments and the tail fan, which may become necrotic and reddened similar to the colour of cooked shrimp. Severely affected shrimp become moribund and mortalities can be instantaneously high and continue for several days. Mortalities from IMN range from 40 to 70% in cultivated *P. vannamei*, and food conversion ratios (FCR) of infected populations increase from normal values of ~ 1.5 to 4.0 or higher.

Shrimp infected with IMNV displaying white muscle

IMNV has been demonstrated to be transmitted through cannibalism. Transmission via water and vertical transmission from broodstock (trans-ovarian or by contamination of the spawn eggs) to progeny is also likely to occur. IMNV may also be transmitted among farms by faeces of seabirds or shrimp carcasses. Outbreaks of IMN with sudden high mortalities may follow stressful events such as capture by cast-net, feeding, sudden changes in salinity or temperature, etc., in early juvenile, juvenile, or adult *P. vannamei* in regions where IMNV is enzootic. IMN can be confirmed by histopathology, using routine haematoxylin-eosin (H&E) stained paraffin sections and demonstrating characteristic coagulative necrosis of striated skeletal muscle fibres, often with marked oedema among affected muscle fibres. IMN may be also rapidly diagnosed using a nested reverse-transcriptase polymerase chain reaction (RT-PCR) method which provides a rapid, sensitive and specific test to detect IMNV in penaeid shrimp. IMNV can be detected by molecular methods like by *in-situ* hybridisation (ISH), nested RT-PCR and real-time RT-PCR are available. The disease can be prevented by stocking with virus free PL produced from IMNV-free broodstock.

2.4 Taura Syndrome (TS)

Taura Syndrome was first identified from farms around the Taura River in Ecuador in 1992 and the disease spread rapidly to the entire region of Latin and North America within three years. Subsequently, TS was also reported from Asia including Mainland China and Taiwan (from 1999), and in late 2003 in Thailand, probably through the regional and international transfer of live PL and broodstock of *P. vannamei*. Initial work suggested that

TS was caused by a toxic pesticide. However, it is now known that a single or perhaps several very closely related strains (mutants) of the *Taura syndrome virus* (TSV) are responsible for the TS. TSV is a single stranded RNA virus of 32 nm size, non-enveloped icosahedrons and more prone to mutations causing more concern. TSV infections occur in juvenile shrimp (0.1-1.5 g body weight) within two to four weeks of stocking ponds and occur largely within the period of a single moult cycle. In the acute phase of the disease, during pre-moult stage, the shrimp are weak, soft-shelled, have empty gut and diffuse expanded chromatophores that appear red, particularly in the tail (hence the common name - red tail disease). Such animals will usually die during moulting (5-95%). Adult shrimp are known to be more resistant than juveniles Shrimp that survive infection show signs of recovery and enter the chronic phase of the disease. Such shrimp show multiple, randomly distributed, irregular, pitted, melanised lesions of the cuticle. These gross lesions will persist, but may be lost during moulting, and the shrimp thereafter appear normal. TS can be diagnosed using standard histological and molecular methods of detection. Specific DNA probes applied to *in situ* hybridization assays with paraffin sections provide the confirmatory diagnosis. Reverse transcriptase polymerase chain reaction (RT-PCR) assay is commonly used for larger sample sizes and non-lethal sampling for broodstock. Histological demonstration of enlarged lymphoid organs (LO) with multiple LO spheroids and multifocal areas of necrosis in the cuticular epithelium of the general body surface, appendages, gills, hindgut, and foregut (the oesophagus, anterior and posterior chambers of the stomach).The mechanism of transmission of TSV can be through contaminated PL and broodstock. Recently it has been shown that mechanical transfer through insect. The disease can be prevented by avoidance of reintroduction of the virus from wild shrimp and carriers and stocking with TSV-free PL produced from TSV-free broodstock.

2.5 Yellow Head Disease (YHD)

Yellow head disease is a major viral disease that caused extensive losses to shrimp farms in Thailand during 1990-91. YHD has been reported in China, Taipei, Indonesia, Malaysia, the Philippines, Sri Lanka, Thailand and Vietnam. Outbreaks of YHD with heavy mortalities have been reported in farmed black tiger shrimp and pacific white shrimp. It is reported to be highly prevalent (>50%) sampled farmed and wild populations in Australia, Asia, East Africa and Mexico. YHD is caused by *Infectious type I yellow head virus* (YHV). YHV is rod shaped enveloped viruses of 40-60 nm by 150-200 nm size, containing single stranded RNA. YHV affects tissues of ectodermal and mesodermal origin including lymphoid organ, haemocytes, haematopoietic tissue, gill lamellae and spongy connective tissue of the subcutis, gut, antennal gland, gonads, nerve tracts and ganglia. YHV principally affects pond reared juvenile stages of 5-15 g. Affected shrimp typically feed voraciously for two to three days and then stop feeding abruptly and are seen swimming near the periphery of the pond. YHV infections can cause swollen and yellow discolouration of hepatopancreas in infected shrimps. YHD can cause up to 100% mortality in infected *P. monodon* ponds within 3-5 days of the first appearance of clinical signs. The primary mechanism of spread of YHV in pond culture appears to be through water and mechanical means. Infected broodstock can pass on the virus to larvae in the maturation/hatchery facilities if thorough disinfection protocols are not strictly adhered to. YHV can be detected by RT-PCR or with a probe designed for dot-blot and *in situ* hybridization tests. It can also be diagnosed histologically in moribund shrimp by the presence of intensely basophilic inclusions, most easily in H&E stained sections of stomach or gill tissue, or simply by rapid fixation and staining of gill tissue and microscopic examination. Methods of YHV eradication in ponds are much the same as for other viruses and involve BMPs that include pond preparation by disinfection and elimination of carriers and production of virus free broodstock and PL for pond stocking.

2.6 *Infection with decapod iridescent virus 1 (DIV1)*

Infection with Decapod Iridescent Virus 1 (DIV1**)** was first isolated and identified in 2017 from Zhejiang Province in China. There have been reports of DIV1 from Thailand at a very low prevalence. Synonyms of infection of DIV1 are infection with shrimp hemocyte iridescent virus (SHIV). Infection with DIV1 has been proposed for listing in the OIE Aquatic Animal Health Code (OIE, 2016). The disease meets the OIE definition of an „emerging disease" and, members must report it in accordance with Article 1.1.4 of the Aquatic Code. Infection with DIV1 is listed in the OIE/NACA quarterly aquatic animal disease reporting programme (https://enaca.org). There are two original isolations of *Decapod iridescent virus 1* (DIV1): Shrimp haemocyte iridescent virus and *Cherax quadricarinatus iridovirus*. DIV1 assigned as the only member of the genus Decapodiridovirus within the *Iridoviridae* family by the International Committee on Taxonomy of Viruses (ICTV). DIV1 infected shrimp display slightly reddish body discolouration, hepatopancreatic atrophy with colour fading, empty stomach and guts and soft shell in partially infected shrimp. The moribund shrimp lose their swimming ability and sank to the pond bottom. The symptoms and mortality are observed in the infected *P. vannamei* from post larvae to sub-adult shrimp stages. Challenge tests with *P. vannamei* via per os and reverse gavage have demonstrated that direct horizontal transmission as an important route of transmission. There is no evidence of vertical transmission; however, samples from hatcheries have been found to be DIV1 positive. The sensitive nested PCR and real-time PCR methods have been established In situ hybridization (ISH) and in situ DIG-labelling-loop-mediated DNA Amplification (ISDL) have been validated. Enhanced biosecurity is the key strategy for control of infection with DIV1, including surveillance of farms, quarantine, and testing for DIV1 in broodstock and post-larvae. Biosecurity measures to minimise fomite spread via equipment, vehicles (i.e. cleaning and disinfection) should also be implemented. Restrictions on the movement of live crustaceans and removal of moribund or dead individuals from affected farms will limit the spread of the disease.

2.7 Viral covert mortality disease (VCMD)

Viral covert mortality disease was first reported in 2009 from China as a serious disease outbreak due to heavy economic loss shrimp aquaculture sector. The disease was commonly named as Covert mortality disease (CMD) due to the moribund shrimp which died at the bottom of the pond. Later CMD was renamed as viral covert mortality disease (VCMD). The cumulative mortality of VCMD was variable, but it reaches 80 to 90% in some *P. vannamei* culture ponds. After the first appearance in China, CMNV was also reported in Thailand, Vietnam and Ecuador. A new emerging virus, *covert mortality nodavirus* (CMNV), was identified to be the aetiological agent of covert mortality disease. CMNV is a non-enveloped, spherical-shaped, single-strand RNA virus, a new member of *Alphanodavirus*. The virus consists of an icosahedral capsid size ranging from 29 to 35 nm in diameter. CMNV-infected shrimp shows hepatopancreatic atrophy and necrosis, empty stomach and gut, soft shell, slow growth of infected shrimp, abdominal muscle whitening and necrosis. The histopathology analysis of CMNV infected shrimp showed coagulative necrosis of striated muscle, eosinophilic inclusions in the epithelium of the tubules in the hepatopancreas and lymphoid organ, mass karyopyknotic nuclei which existed in the muscle and lymphoid organ and the tubular epithelium of the hepatopancreas with significant atrophy. Experimental challenge by injection mode showed reproduction of VCMD confirmation of the causative pathogen. Currently, the detection of CMNV include a reverse transcription nested PCR (RT-nPCR) and real-time reverse transcription PCR (qRT-PCR), quantitative reverse transcription loop-mediated isothermal amplification (qRT-LAMP) assay and fluorescence in situ hybridization (FISH) methods. Farmers in the shrimp aquaculture need to pay close attention and takemeasures to prevent disease outbreaks by practicing BMPs.

2.8. Penaeus vannamei nodavirus (PvNV) infection

The disease was first reported in cultured *Penaeus vannamei* in Belize in 2004 and in Guayas Province, Ecuador in 2006. The causative agent is a positive single stranded RNA virus, named as *Penaeus vannamei nodavirus* (*Pv*NV) and is related to *Mr*NV belong to *Nodiviridae*. The virus in has been detected in adult shrimps. Affected shrimp exhibit clinical signs, white, opaque lesions in the tail muscle that causes muscle necrosis. Histological examination reveals multifocal necrosis and hemocytic fibrosis in the skeletal muscle and basophilic, intra cytoplasmic inclusions in striated muscle, lymphoid organ and connective tissues. It appears to affect survival in grow out ponds. The disease is associated with environmental stress, such as crowding and high temperature. When the shrimp are stocked at a high density (>50 m–2), or when the temperature is >32°C, survival decreases by 40% in *Pv*NV-infected ponds. It was estimated 50% production loss due to of *Pv*NV in 2004 for the infected farm in Belize. *In situ* hybridization method and a nested RT-PCR assay specific for *Pv*NV have been developed. Also commercial IQ2000RT –PCR diagnostic kit is available for routine screening. The infection is need to be monitored, otherwise it may lead to spread of *Pv*NV to other farms and could result in significant production losses in infected areas.

3. Bacterial diseases in shrimp

3.1 Acute haepatopancreatic necrosis disease (AHPND)

In recent years, acute hepatopancreatic necrosis disease (AHPND) emerged as a major threat to the shrimp industry worldwide. The first outbreak of AHPND occurred in 2009 in China. Since then, outbreaks of the disease have been reported in Malaysia, Thailand, Philippines, Vietnam, Mexico, Bangladesh and USA. So far, there is no report of AHPND from India. The disease caused alarming mortalities and significant economic losses in shrimp industries in many Asian countries. In 2013, the causative agent of AHPND was identified as *V. parahaemolyticus*. Subsequently, it was reported that disease is caused by only those strains which carry pVA1 plasmid and Photorhabdus insect-related (Pir) toxin, PirA and PirB. This virulence plasmid had a size of ~69 kbp. Later on the pVA1 plasmid was reported in several other species such as *V. campbellii, V. owensii, V. harveyi* and *V. punensis*. *V. parahaemolyticus* causing AHPND is known to initially colonize the stomach of infected shrimp. Post colonization, the pirAB toxin produced by these bacteria reaches the hepatopancreas and induces sloughing of the tubular epithelial cells. The extensive damage induced by the toxin let the hepatopancreas get atrophied and becoming pale. PCR-based amplification PirAB toxin regions are presently used as a reliable diagnostic tool for AHPND.

3.2. Vibriosis

Apart from AHPND-causing *V. parahaemolyticus*, there are several other species of *Vibrio*, which can affect shrimp. These include *V. parahaemolyticus, V. alginolyticus, V. mimicus, V. harveyi, V. fischeri, V. litoralis, V. metschnikovii* etc. Water bodies of brackishwater aquaculture systems are generally normal habitats of different species of *Vibrio*. However, they also act as opportunistic pathogens of cultured shrimp and affect shrimps during different environmental stress factors including mechanical injury, higher salinity, increased level of ammonia, nitrite and nitrate, low dissolved oxygen, higher stocking density, sudden change of pH, etc. Vibriosis is considered as major problem in hatchery level. In grow-out culture, this disease is characterized by melanised nodules in the gills, opacity of muscle, red discolouration of the appendages etc. Gathering at edge of the ponds is also commonly observed. Haemolymph of the affected shrimp does not clot or clot at very slow rate. Different species of *Vibrio* can be isolated from haemolymph and hepatopancreas by plating on TCBS agar and Zobell marine agar. The luminescence can be demonstrated by preparing a smear from haemolymph and observation in dark. In case of heavy infection, the luminescence can be seen if the haemolymph is observed in dark. Some species of Vibrio also cause septic hepatopancreatic necrosis causing necrosis of hepatopancreas.

3.4. Necrotizing hepatopancreatitis (NHP)

Necrotizing hepatopancreatitis is caused by a rickettsia like bacterium called as *Hepatobacter penaei*, which infects the hepatopancreas of shrimp. The clinical signs of this disease include lethargy, emaciation, soft shells, heavy fouling from external parasites, black gills and reduced growth. The hepatopancreas appears pale to white. NHP requires lengthy periods of high air temperature (29°–31°C) and elevated salinity (20–40 ppt). Mortality can be 90–95% within 30 days of an outbreak. Mortalities usually occur midway through the grow-out phase. NHP appears to be transmitted by direct ingestion of an unidentified carrier (a reservoir host). Diagnosis is generally done by PCR. Histopathology of hepatopancreas shows empty hepatopancreatic tubule with capsule formation. Cell sloughing, tubular atrophy, melanization and formation of multifocal haemocytic capsules are also observed. In some cases, the cluster of intra-cytoplasmic bacteria is also observed.

3.5. *Shell disease and necrosis of appendages in crustaceans*

Shell disease commonly affects the exoskeleton of shrimp larvae. It is also known as chitinolytic bacterial disease, brown spot disease, black spot disease and rust disease. It is often associated with *Vibrio* spp, *Aeromonas*, and *Pseudomonas*. The shell disease is identified by the presence of black spots and lesions on the exoskeleton and appendages. The progressive erosion of lesions leads to the aggressive multiplication of bacteria and pathogens in the affected region. Severe infection results in the loss of an affected appendage and the exoskeleton ultimately hampered the molting and locomotion. Thus the affected larvae become susceptible, weak, and eventually, mortality occurs due to stress/ energy exhaustion. In tail necrosis the affected shrimp had necrosis of uropods and pleopods. In acute case of infection the uropods were completely lost; the muscle in the distal portion of the abdomen became completely necrotic and began to decompose. The diseased shrimp lost their swimming ability and exhibited erratic gliding movements near the edge of ponds (Jayasree et al., 2006). There is no known treatment for shell disease. The management of shell disease can be achieved by maintaining organic load at a low level, by removing dead larvae, sediments, and debris, which may harbour a heavy load of bacteria. High stocking may be avoided, and good water quality and proper diet should be maintained.

Shrimp affected with (A) blackening of gills (B) tail rot (C) rotting of pleopod

Adoption of better management practices (BMPs), maintaining good water quality, providing optimum feed, adopting biosecurity measures using specific pathogen free (SPF) could serve to combat diseases. As antibiotic application is not a viable options in shrimp culture a search for alternative therapeutic such as phage therapy and multivalent vaccine could be an effective control measures against bacterial infections. In case of treatment of any shrimp disease, it is better to apply any chemical or medicine only after consultation with aquaculture experts or fish heal professional. Indiscriminate use of any chemical or medicine may adversely affect the diseased condition. Moreover, accidental use of any banned chemical or substances may result in export rejection leading to heavy economic loss.

*4. **Hepatopancreatic microsporidiosis (HPM)***

Hepatopancreatic microsporidiasis (HPM) is a disease caused by an emerging shrimp microsporidian *Enterocytozoon hepatopenaei* (EHP). Since the target organ of this microsporidian is the hepatopancreas, the disease was named as hepatopancreatic microsporidiasis. The parasite was first recorded in *P. monodon* affected by growth retardation in Thailand. Subsequently, it was identified and characterized in Thailand in 2009. Thereafter, its spread was recorded in many shrimp farming nations including south-east Asian countries and Latin America. So far, EHP infection has been reported in cultured *P. monodon and P. vannamei*, and is suspected to occur in *P. japonicus* as well. There are no distinct reported visual clinical signs for EHP infection in shrimp farms but it is reported to be associated with retarded growth and white faeces syndrome (WFS). Infection in the hepatopancreas may cause malabsorption of nutrients and result in growth retardation. In India, the disease was first recorded in 2016. Since then, EHP epizootics and spread was severe in the east coast of India. Recently, the disease transmission has been recorded in the west coast of India and inland saline areas in Haryana. In India, the probability of occurrence of this disease during the period 2018-19 in 7259 ha of shrimp farming area in 23 coastal districts was reported as 17%. The production loss due to the disease during the period 2018-19 was also calculated as 0.77 million tonnes with a revenue loss of US$ 567.62 million. There are no known distinctive visual clinical signs of an EHP infection, but it is often associated with stunted growth. Hence, an EHP infection may be suspected when unusual retarded growth in the absence of other gross signs is observed. Affected shrimps exhibit slow growth, reduced feeding, and loss of appetite. Severe infection by EHP in shrimp farms can increase the susceptibility to other bacterial infections (Vibrio spp.) and result in mortality. In shrimp farms, EHP does not cause mass mortality. Shrimps in affected farms show size variation, and suffer low level daily mortality. The feed conversion efficiency was drastically affected resulting in severe economic loss to the farmers. EHP is transmitted horizontally by cohabitation and cannibalism. In cohabitation, healthy shrimps ingest (faecal-oral route) spores released into the water by infected shrimps. In cannibalism, healthy shrimps are infected by feeding (oral route) the infected moribund or dead shrimps. Vertical transmission of EHP to the offspring from infected broodstock is largely unknown. So far, EHP has not been found in the ovaries and testes of EHP infected shrimps. At this stage, the possibility of vertical transmission of EHP may not be ruled out. Still, the infected female brooders may transmit the infection to the larvae passively through faeces.

EHP infection can be detected by demonstrating spores (1.1 ± 0.2 μm × 0.6 ± 0.2 μm) in hepatopancreatic tissue sections and faecal matter. Spores can be viewed by light microscopy in a smear, squash preparation of hepatopancreas, and faecal matter stained with specific stains such as Giemsa, Phloxin, trichrome, and Calcofluor white Stain. The spores can also be demonstrated in histological preparation of hepatopancreas tissue sections stained with Eosin and hematoxylin. Modified trichrome stain (Ryan-blue method) was used for the microscopic detection of EHP. More specific molecular diagnostic tests such as Polymerase chain reaction (PCR), Real-time PCR, *In situ* hybridization (ISH), Loop-mediated isothermal amplification (LAMP), Recombinase polymerase amplification (RPA) and CRISPR based diagnostics are also available for the detection of EHP. The PCR targeting small subunit ribosomal RNA (SSU rRNA) gene and more specific spore wall protein gene (SWP) and β-tubulin gene are available for the diagnosis of EHP. Real-time PCR assays based on the use of SYBR I green and the Taqman probe for the quantification and diagnosis of EHP have been reported. If a pond is affected by EHP then, the following pond soil treatment is suggested for subsequent crop: The spores of EHP have thick walls and are not easy to inactivate. Even high levels of chlorine alone are not effective.For pond sediment treatment apply CaO (quick lime, burnt lime,unslaked lime, or hot lime) @ 6 ton/ha. Plough the CaO into the dry pond sediment (10-12 cm) and then moisten the sediment to activate the lime. Leave for one week before drying or filling. After application of CaO, the soil pH should rise to 12 or more for a couple of days and then to the normal range as it absorbs carbon dioxide and forms CaCO3. Hatcheries should test fresh feeds and artemia by PCR method. Stock PCR negative seed, healthy and strong seed. Stock seed in ponds with good plankton/bloom.

5. Disease syndromes in shrimp culture

5.1. White faeces syndrome (WFS)

WFS has recently been considered 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. WFS usually occur after 50-60 days of culture (DOC). Ponds affected with this disease 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. There are multiple causes for WFS, which may be associated with presence of vermiform like gregarine bodies, vibriosis, *Enterocytozoan hepatopenaei*, blue green algae and loose shell syndrome. On bacteriological examination of haemolymph, a very high load of *Vibrio* is generally observed in WFS infected shrimp. Six species of fungi (*Aspergillus flavus, A. ochraceus, A. japonicus, Penicillium* spp., *Fusarium* spp., and *Cladosporium cladosporioides*) were isolated from shrimp naturally infected with WFS. Reduced stocking density, proper water exchange together with better management practices will be helpful in evading WFS. Application of gut probiotics at regular interval can also prevent the onset of this disease.

5.2. Black gill disease

This disease generally occurs when plankton content of water is too high. Poor pond bottom quality, low dissolved oxygen content, poor water quality parameters and high stocking density are also the causal factors of this disease. This is also known fouling disease. Gill becomes black in colour and is generally colonized with different saprophytic bacteria (*Flavobacterium, Cytophaga*, etc.) and parasite (e.g., *Zoothamnium* spp.). Addition of lime (quantity depends on pH), water exchange and increase of duration of aeration may help in controlling this disease. This disease is very often associated with deficiency of Vitamin C. To avoid this disease condition, the shrimp must not be overfed.

Black gill disease in shrimp

5.3. Loose shell syndrome (LSS)

This disease also causes a heavy economic loss among the shrimp farmers and was first reported in India in 1998. The incidence is more in summer than in winter. In India, the disease is more prevalent in some districts of Andhra Pradesh (East Godavari, West Godavari and Nellore) and Tamil Nadu. The disease is characterized by spongy abdomen due to muscular dystrophy, shrunken hepatopancreas and poor meat quality, which generally fetch reduced market price. The affected shrimps cannot moult and the gap between muscle and shell is generally increased with accumulation of water. The etiology of this disease is still not confirmed. Different species of *Vibrio* has been isolated from affected shrimp. The involvement of a filterable infectious viral agent has also been suspected for the disease. Maintenance of good aquaculture practices including water quality parameters and adaption of strict biosecurity measures may be of help in controlling this disease. Recently, it is also observed that sometimes high nitrite content of pond water is associated with loose and weak shell.

Shrimp with LSS condition

5.4 White muscle syndrome (WMS)

White muscle syndrome (WMS) was first reported from Ecuador associated with muscle necrosis and low mortalities in the *P. vannamei* grow-out cultures. The WMS-affected shrimp show lesions focal to extensive necrosis in striated muscle tissues, exhibiting a white, opaque appearance in abdominal area. Similar lesions have been described in shrimp infected with Infectious myonecrosis (IMN), penaeid white tail disease (PWTD). From various studies WMS in shrimp can also be triggered by the progressive infection of microsporidians belonging to the genera *Ameson* and *Agmasoma*, dietary deficiency of selenium and sudden variations in water quality parameters like salinity, temperature and dissolved oxygen. Histologically, WMS-affected shrimp samples with macroscopic lesions revealed destruction of sarcomere structure, coagulative muscle necrosis and haemocytic infiltration. Though histological lesions seen in the suspected sample are indistinguishable from those reported for *Infectious myonecrosis virus* (IMNV) and PWTD in *P. vannamei,* absence of cytoplasmic inclusions in the skeletal muscle is characteristic feature of WMS. As WMS-affected samples were devoid of IMNV and *Pv*NV infections, it was suggested that the aetiological agent of WMS could be either a novel infectious agent or a variant strain of IMNV.

White, opaque necrotic patchy areas in abdominal muscle seen in WMS-affected farmed shrimps

5.5 Running mortality syndrome (RMS)

Running mortality syndrome (RMS) is an idiopathic syndrome that causes constant low-level mortalities on daily basis throughout the culture period, hence the name running mortality syndrome (RMS) was commonly identified by the shrimp farmers in India. Since 2011, RMS is extensively reported in the *P. vannamei* farming areas in Andhra Pradesh (AP) and Tamil Nadu (TN) from India. The only apparent clinical sign of RMS is the presence of whitish patches in the 2^{nd} to 4^{th} abdominal segments of affected shrimp was reported (Alavandi et al., 2019). Animals affected with RMS were reported to be free from known pathogens of shrimp viruses such as WSSV, IHHNV, IMNV, TSV, YHV, MBV, HPV, and PvNV. However, *Vibrio* spp., *viz., V. parahaemolyticus* and *V. azureus* are predominantly observed in haemolymph of RMS-affected shrimp. In RMS-affected farms, usually daily mortalities are reported from 30 - 40 days of culture (DOC). Though the mortality due to RMS was slow (< 1%/day), the cumulative damage was reported to be as high as 70% in some cases, hence farmers opt for an early or premature harvesting leading to severe economic loss. The muscle tissue had severe necrosis accompanied by haemocytic infiltration and hepatopancreas from most of the affected animals appears normal on histopathological examination. However, some of the hepatopancreas samples of RMS-affected shrimp showed increased inter-hepatopancreatic tubular space with haemocytic infiltration and enlargement of the nucleus. Experiments on oral feeding and cohabitation with the infected animals failed to reproduce of RMS condition. RMS-affected shrimp exhibited recovery, appeared healthy and active after 6-7 days with optimal water parameters when introduced in laboratory condition for monitoring. Though the exact cause of RMS is unidentified, the condition is reported to be linked to high stocking densities, high nitrite-N, and high turbidity. Accordingly, it is stated that RMS is a pond management-associated syndrome and can be prevented by best management practices.

6. General health management

SPF shrimp are expected to be free from the viral pathogens which are known to cause major losses to the shrimp aquaculture, including WSSV, YHV, TSV, IHHNV and IMNV. SPF refers only to the present pathogen status for specific pathogens and not to pathogen resistance or future pathogen status. SPF means that these animals will not suffer from diseases caused by specified diseases for which the animal is declared "free" when cultured under „strict‟ biosecurity. However, it does not guarantee against these shrimp getting infected with unknown pathogens or known pathogens which are not screened. Further, the SPF shrimp are not resistant to pathogens and these shrimp can become infected by any pathogen that they encounter during culture. Focusing efforts are needed on producing high quality seed, following farm biosecurity, better pond management to reduce stress and risk of infection.

7. Conclusion

Aquaculture is vital to the economies of several countries. Growing demand for seafood and limitations on production from capture fisheries will inevitably lead to the increased intensification and commercialization of shrimp aquaculture. This consequently leads to emergence of both known and novel diseases. The occurrence of infectious disease is usually related to a series of happenings involving the interactions between the host, the environment and the presence of pathogens. Enhanced better management practices aid in preventing the diseases epidemics. The disease emergence and its spread are due to the various linked events involving the interactions between the host, the environment and the pathogens. It would be always cautious to monitor and encourage producers to consider standard management strategies such as the lowering of stocking densities, maintaining a good water quality, and the use of disease-free shrimp PLs for stocking, improved better management practices, routine farm biosecurity measures and responsible trade practices to prevent epizootics in aquaculture. Shrimp health is a shared responsibility, of every stakeholder's contribution to minimize losses and sustain productivity**.**

Bibliography

1. AftabUddin, *et al.* (2018).First incidence of loose-shell syndrome disease in the giant tiger shrimp *Penaeus monodon* from the brackish water ponds in Bangladesh, Journal of Applied Animal Research, 46:1, 210-217
2. Dey, B.K., Dugassa, G.H., Hinzano, S.M and Bossier, P. 2019. Causative agent, diagnosis and management of white spot disease in shrimp: a Review. *Reviews in Aquaculture* 12(2): 822-865.
3. Frischer M. E. et al. (2018). Pathology, effect and transmission of Black gill in commercial Penaeid shrimp from the South Atlantic Bight. Journal of Shellfish Research, vol.37, Issue 1, 149-158.
4. Gooding, E.L., Kendrick, M.R., Brunson, J.F., Kingsley-Smith, P.R., Fowler, A.E., Frischer, M.E and Byers, J.E. 2020. Black gill increases the susceptibility of white shrimp, *Penaeus setiferus* (Linnaeus, 1767), to common estuarine predators. Journal of Experimental Marine Biology and Ecology. 524, 151284.
5. Hou, D. *et al.* (2018). Intestinal bacterial signatures of white feces syndrome in shrimp. *Appl Microbiol Biotechnol* **102,** 3701–3709. https://doi.org/10.1007/s00253-018-8855-2
6. OIE., (2021). Aquatic manual online access. https://www.oie.int/en/whatwe-
7. do/standards/codes-and-manuals/aquatic-manual-online-access/ Accessed February 25, 2022.
8. Qiu, L., Chen, M-M., Wan, X-Y., Li, C., Zhang, Q-L., Wang, R-Y., Cheng, DY., Dong, X., Yang, B., Wang, S-H., Xiang, J-H. and Huang, J. (2017). Characterization of a new member of Iridoviridae, Shrimp hemocyte iridescent virus (SHIV), found in white leg shrimp (*Litopenaeus vannamei*). *Scientific reports,* **7:** 1-13.
9. Chaijarasphong, T., Munkongwongsiri, N., Stentiford, G.D., Aldama-Cano, D.J., Thansa, K., Flegel, T.W., Sritunyalucksana, K. and Itsathitphaisarn, O., 2020. The shrimp microsporidian *Enterocytozoon hepatopenaei* (EHP): Biology, pathology, diagnostics and control. Journal of Invertebrate Pathology, p.107458.
10. Tourtip, S., Wongtripop, S., Stentiford, G.D., Bateman, K.S., Sriurairatana, S., Chavadej, J., Sritunyalucksana, K. and Withyachumnarnkul, B., 2009.

Enterocytozoon hepatopenaei sp. nov.(Microsporida: Enterocytozoonidae), a parasite of the black tiger shrimp Penaeus monodon (Decapoda: Penaeidae): Fine structure and phylogenetic relationships. Journal of invertebrate pathology, 102(1), pp.21-29.

11. Zhao, R.H., Gao, W., Qiu, L., Chen, X., Dong, X., Li, C. and Huang, J., 2020. A staining method for detection of *Enterocytozoon hepatopenaei* (EHP) spores with calcofluor white. Journal of invertebrate pathology, 172, p.107347.

12. Zhang, Q., Liu, Q., Liu, S., Yang, H., Liu, S., Zhu, L., Yang, B., Jin, J. Ding, L., Wang, X., Liang, Y., Wang, Q. and Huang, J. (2014). A new nodavirus is associated with covert mortality disease of shrimp. *Journal of General Virology,* **95:** 2700-2709.

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Application of probiotics and immunostimulants in *Penaeus vannamei* farming

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Aquaculture is an important economic activity of India providing livelihood, generating employment and earning export revenue to the country. Sector is facing economic loss due to deteriorating environmental conditions and stressful conditions leading to diseases and poor growth in intensive aquaculture operations. Prevention and control of disease is the key for successful farming operations. Probiotics protect against disease by activating both cellular and humoral immune defences by increasing phagocytosis, respiratory burst and antibacterial activity in aquatic animals. Lactic acid bacteria are known to produce compounds such as bacteriocins that inhibit the growth of other microorganisms.

1. Probiotics

The probiotics for aquaculture are defined as live microbes with beneficial effect on fish/ shrimp or improving the quality of pond environment" Probiotics maintain the healthy balance between the healthy and pathogenic micboes in the culture environment. Probiotic play important role in aquaculture particularly in improving productivity, nutrient utilization, disease control, water quality and the impact of discharge on the surrounding environment. Bacteria most commonly present in the probiotics for use in aquaculture include, Lactobacillus, *Bacillus* spp., photosynthetic bacteria and yeast. Probiotics are the best alternative to chemicals and antibiotics in aquaculture. Generally the probiotics are expected to perform in wide variety of culture environments, freshwater, brackishwater and marine in addition to varying pH, DO, turbidity etc. The probiotic formulations used in aquaculture are classified as:

Gut probiotics: applied through feed improve the health by improving digestion, immune response to diseases, resistance to stress and the common microbes are *Bacillus* spp.

Water probiotics: broadcasted on the water surface improve the water quality through controlling Total Ammonia Nitrogen (TAN) and other nitrogenous metabolic wastes. The common microbes are, *Nitrobacter* spp. *Nitrosomonas* spp.

Soil probiotics: applied at the pond bottom to control toxic sulphur molecules. The commonly used microbes are Thiobacillus, spp.

2. Mechanisms of action of probiotics

- production of compounds that are inhibitory to pathogens
- competing with pathogens for nutritional requirements and adhesion sites
- improving the digestibility through the production of enzymes and supplementation of
- micronutrients
- enhancing host immunity against both biotic and abiotic stresses
- improving the culture environment through bioremediation of metabolic toxins and
- interaction with phytoplankton
- clean up toxic metabolites through bioremediation
- degrade, transform or chelate various toxic chemicals

3. Is the intestinal environment of aquatic animals favourable to probiotics?

Since shrimp larvae are released directly into the environment, microbes in the water directly attack the young ones. Hence, the beneficial microbes are very much essential to protect the larvae at the early developmental stages.

4. Application of probiotics to improve water quality

Mechanism of probiotics to controlling pathogenic bacteria is called biocontrol and the one improving the water quality is called bioremediation. Floating biofilters pre-inoculated with nitrifying bacteria decreased the amounts of ammonia and nitrite in the rearing water. *Bacillus* spp. formulations applied near pond aerators reduced chemical oxygen demand. Probiotics when applied to pond water reduce the levels of pathogenic *Vibrio* sp. Degradation of organic matter by *Bacillus* spp. improves water quality.

4.1. Antagonism to pathogens

Probiotic bacteria inhibit the pathogenic bacteria by production of antibiotics and inhibitory substances, like, organic acids, hydrogen peroxide and siderophores.

4.2. Intestinal colonization

Gut probiotics, both bacteria and yeast, act by colonizing the host gut and also by transient presence when applied in high concentration.

4.3. Protection against pathogen challenge

Administration of probiotic bacteria confers protection against the pathogen challenge; improve survival in early life stages of shrimp. This protection could be due to production of inhibitory substances that blocked bacterial growth.

4.4. Source of nutrients and enzymatic contribution to digestion

Probiotics benefit the host digestive process by supplying fatty acids and vitamins, extracellular enzymes, such as proteases, lipases, some growth factors.

5. Methods of probiotics application

- addition via live food
- bathing/immersion
- addition to culture water
- addition to formulated feed

6. Basic characteristics of a microbe in probiotic formulation

- Safe to the host and its environment
- Ability to administer through ingestion colonization and proliferation within the host

Generally the probiotic containing multi-strains are much more effective than the single strain formulations. Probiotics are highly effective when applied in combination with prebiotics and immune stimulating agents. Enrichment of live feeds like, artemia, rotifier, copepods with probiotics as encapsulations is best practice in hatcheries. Probiotics increase the levels of lysozyme and respiratory burst, phagocytic and complement activity. Application of probiotics in appropriate dose is very important as lower doses may be ineffective and high dose may lead to unnecessary expenditure without additional benefits. Application of probiotic formulations in several short spells is more effective than a single long duration. Prolonged application of probiotic formulations may lead to cost escalation without much benefit sometimes may also lead to immune suppression. Feeding probiotics in repeats of short-term-cycles gives higher benefits.

6.1. Enhance immune responses

Application of probiotics is known to increase leucocytes, lymphocytes, monocytes, erythrocytes, neutrophil adherence, migration of neutrophils and plasma bactericidal activity, complement activity, cytotoxicity, phagocytic and superoxide dismutase activities, total globulin, albumin, serum bacterial agglutination titres, serum peroxidase and blood respiratory burst activities, phagocytic activity, lysozymes, respiratory burst, antiprotease activity, peroxidase activity .

Important criteria for selecting a probiotic product for application in aquaculture

- complete name of the microbes in the product
- strength of the product in terms of copy number (cfu/g)
- mode of application (through, feed, water or sand)
- combination of water and soil probiotic may be allowed but not themixture with gut probiotics

6.2. Probiotics improve water quality

Probiotics have proven their effectiveness in improving water quality in different approaches. They enhanced decomposition of organic matter, reduced nitrogen and phosphorus concentrations, and controlled ammonia, nitrite, and hydrogen sulphide. Reduce organic matter accumulation, mitigated nitrogen and phosphate pollution in the sediments, reduced metabolic wastes during transportation, reducing a number of pathogenic bacteria. Probiotics improved digestibility: Administration of probiotics enhance the digestive enzymes like, alginate lyases, amylases and proteases carbohydrolases and lipases, generation of essential nutrients such as fatty acids, biotin and vitamin B12.

6.3. Specific probiotic species

There is no one good formulation for all purpose; farmers need to standardize the formulations based on the requirement mostly based on trial and error basis.

7. Limitation of probiotic effectiveness

Since microbes are very much sensitive to environmental parameters it is not necessary that products work effectively in different culture systems. Since the beneficial effect of the probiotic bacteria depends its interaction with host it is not necessary that probiotics work effectively in different cultured animals.Since probiotics needs to be build up in the system for effective action regular application of the probiotic products ensures the beneficial effect. Since different bacterial compositions work in different site of actions like gut, water and soil beneficial effect of the product can be achieved only when applied in appropriate route. Since there will be a completion between the opportunistic pathogen and the probiotic bacteria it is necessary that probiotic product needs to be applied in sufficient quantity.

8. Prebiotics

Prebiotics are the non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of probiotic bacteria in the gut and thus improves host health. Ex. Fructooligosaccharides, lactosucrose etc.

9. Immunostimulants

Immunostimulants are naturally occurring compound that modulates the immune system by increasing the hosts‟ resistance against diseases‟ . Enhancing the disease resistance power of the host is one of the crucial approaches for preventing diseases. Several substances of natural, chemical and microbial origins have been known to possess‟ immune stimulating potential. Though the administration of immune-stimulating agents for humans and animals‟ general health and well-being is a well-established practice, its importance has been recognized only recently in aquaculture. The development of immune stimulants for use in aquatic organisms was slow due to the lack of understanding of the basic immunology and the efficient parameters to evaluate the immune response in crustaceans and molluscs. The information on the molecular immune system of aquatic invertebrates and tools for assessing the immune reactive molecules is emerging. This has made it possible to develop new molecules for effective stimulation of the immune system of aquatic organisms and the corresponding resistance to invading pathogens. Similar to human and domestic animals, vaccination is the best possible disease protection method. However, the practical feasibility of this approach in aquaculture has led to the development of immune stimulating agents. Common immune stimulating agents include, polysaccharides, vitamins, different components of bacteria, biologically active materials, herbal and synthetic drugs.

Classification of immune stimulating agents based on

1. Their origin
2. Mode of action
3. Mode of administrated

9.1. Immune stimulation in crustaceans

Since the invertebrate immune system is less developed the immune stimulants act in crustaceans by increasing the phagocytosis of pathogens by activating phagocytic cells in the hemolymph; increase the antibacterial and antiseptic properties of hemolymph, activate the prophenoloxidase system, mediate signal recognition and phagocytosis expression of immunostimulant genes.

9.2. Clinical observations of immune stimulating agents

Improved growth rates, survival rates and disease resistance; immunostimulants are safer alternative to antibiotic to control bacterial infections and chemicals for managing the environmental stress

10. Conclusion and perspectives

The usage of antibiotics and chemotherapeutics in aquaculture and their subsequent undesirable consequences have made the researchers think about the safe alternative – probiotics and immune stimulants. Owing to the residual and side effects, antibiotics cannot be used in aquaculture industry, but probiotics and immunostimulants can be used to protect the crop against the abiotic and biotic stress conditions. Multiple bioactivities produced by probiotics and as components of immune stimulants help improving the immunity of aquatic animals. Use of these compounds plays a key role in promoting economically viable and environmentally sustainable aquaculture.

Bibliography

1. Hai, N.V. (2015). The use of probiotics in aquaculture. *Journal of Applied Microbiology*, **119:** 917-935.
2. Pandiyan, P., Balaraman, D., Thirunavukkarasu, R., George, E.G.J., Subaramaniyan, K., Manikkam, S. and Sadayappan, B. (2013). Probiotics in aquaculture. *Drug invention today*, **5:** 55-59.
3. Pérez-Sánchez, T., Ruiz-Zarzuela, I., de Blas, I. and Balcázar, J.L. (2014). Probiotics in aquaculture: a current assessment. *Reviews in Aquaculture*, **6:** 133-146.
4. Wang, W., Sun, J., Liu, C. and Xue, Z. (2017). Application of immunostimulants in aquaculture: current knowledge and future perspectives. *Aquaculture Research*, **48:** 1-23.

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Biosecurity and Quarantine Measures for Aquaculture Health Management

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1. Introduction

The nutritional requirement of large world population is compensated by fish and fishery products to a great extent as these are considered as the source for cheap animal protein. As per the current trend, the production from capture fisheries has been continuously declining and therefore aquaculture can be considered as the future source to meet with the increasing demand for fish. Brackishwater ecosystem in coastal zones are potential source as these are considered as the zero foot print because the water is not suitable for drinking, agriculture or construction and therefore very appropriate for aquaculture practice. Among all the aquatic animals, crustaceans contribute significantly to the total fish production. Based on the recent observation, crustaceans alone contribute more than 9 million tons of the total more than 114 million tons of aquaculture production.

Among the crustaceans, shrimps forms a major share as shrimps are synonym to crustacean culture which contributes more than 60% of the total production. Shrimp aquaculture has been continuously evolving and considered as a lucrative seafood industry because of its high export value from its short rearing period. Shrimp form almost 22% of the total global international trade of fishery products. India has been in the forefront as far as shrimp aquaculture is concerned. India earns almost $ 4 billion from the shrimp export which is about 16% of global export. During the recent times, farmers are adopting modern culture practices such as biofloc and raceway culture and thereby there has been a continuous increase in production and export value.

In spite of the steady progress in production, shrimp aquaculture has continuously been threatened by disease related crop loss. Disease appears to be the prime obstacle for the smooth progress of shrimp aquaculture industry. Disease continues to remain as the primary issue for farmers for the past several years based on Global Aquaculture Alliance (GAA) survey report. Frequent appearance of several existing and emerging diseases brings huge economic loss to the farmers. Globally more than 1 billion US $ disease related annual loss in shrimp aquaculture has been estimated.

Similar to other countries, India also continuously records disease related crop loss in shrimp aquaculture and as per the recent estimation it can be more than 6000 crores Indian rupees per annum. India has a huge potential for brackishwater aquaculture and still a major chunk of it remains unexploited. With years to come, it is highly expected that there will be increase in effort to explore and utilise this unexploited treasure land. At the same time, there should be proper planning and protocols in hand to counteract all the associated risk factors.

To minimize the introduction and spreading of infectious diseases, a set of well-designed practices, called as Biosecurity need to be followed.

Through the adoption of biosecurity, animals can avoid the unnecessary burden of several stress factors and mortality and thereby avoid huge economic loss to the shrimp farmers. In a well-designed biosecurity protocol, it is highly essential to accurately determine the specific points of production system that favours the introduction and spread of pathogens.

Once the specific points are determined, it will then be possible to bring corrective measures to check disease occurrence and avoid untimely losses.

Quarantine is a standard protocol to isolate individual/group of organisms, conduct all the necessary tests and ensure existing/new pathogen entry to the point of release. Like biosecurity, this is also an important step in aquaculture practice to avoid disease outbreak and prevent loss. Both biosecurity and quarantine are very much essential for a sustainable aquaculture practice.

2. Biosecurity measures for Brackishwater Aquaculture

Biosecurity is a broad concept and the application of biosecurity concepts to shrimp aquaculture will contribute significantly to reduce losses due to diseases and make this sector more sustainable and environmentally responsible. Implementation of biosecurity practices is an increasingly pressing issue for aquaculture managers, considering the importance of this sector in terms of food security and economic development of the people. Biosecurity measures implemented appropriately can be a cost- effective way of managing disease risks. At farm level, implementing biosecurity plan requires mainly preventive measures at pond / farm level include proper pond preparation to eliminate pathogens and their carriers, treatment of water in reservoirs to inactivate free viruses and kill virus carriers, water filtration using fine filters to keep carriers out, closed zero-water exchange systems to avoid contamination from source water. Shrimp ponds with a history of disease outbreaks have a greater likelihood of future disease outbreaks, and hence, special attention is required during pond preparation. In order to design and develop successful biosecurity protocols, initial determination of specific point of entry of pathogens and their spreading possibilities are very essential and these two points are considered as the major steps. These points will vary from case to case which can be as given below.

Aquatic organisms considered for culture practice

Disease tolerance and infection status of animals selected for culture practice varies from species to species or even to strains. Further, this will also vary based on life stages selected for stocking, initial health status during stocking and maturity of immune system of the stocking animals to provide disease resistance.

Aquatic environment where animals are cultured

Depending on the source water selected for culture practice, initial pathogen load and bacterial diversity and water quality, disease appearance and spread will vary. Also how the water is being maintained during the culture practice and the type of culture practice adopted decides about types of biosecurity need to be practiced.

Type, nature and characteristics of pathogen

Basic biology and life cycle of the pathogen and their survival strategy in the environment (free living state, ability to form spore, ability to survive on inanimate objects, adopting a carrier etc.) determines the criteria to develop biosecurity protocols.

People involved with aquaculture practice (Directly or indirectly)

This includes management staff, workers and visitors. This entirely depends on the nature and education status of all the peoples (how much they understand the principle and how they follow it) Once the above points are decided, maintenance of biosecurity in aquaculture can mainly aims at managing and maintaining the followings to avoid the occurrence and spread of diseases.

Animal Management

While selecting seeds from a hatchery, their pathogen free status should be accurately determined to ensure no pathogens can enter from the hatchery to the culture ponds. The next criteria is to select healthy larvae for which established stress test should be carried out on a sample of the lot from where larvae will be taken for the stocking. Visual and microscopic observation should also be carried out to determine the health status. Wherever possible, it should be tried to get specific pathogen free (SPF) or specific pathogen resistant (SPR) stocks. Maximum numbers of samples of larvae should be tested for the presence of pathogens before packing through a well-established diagnostic protocol. If SPF/SPR stocks are imported, these should pass through proper quarantine for the detection of all possible pathogens.Care should be taken to avoid importing larvae/broodstock from areas known to be affected with specific diseases. For stocking, the concept of "all-in-all-out" should be followed. This means a single batch or group of animals from a single source should be stocked till harvest and any additional stoking during the culture period should be avoided to the maximum possible extent.

Environment management

Starting from the pond preparation it includes several aspects. Pond design and construction should be appropriately done based on the requirement if new ponds are constructed. Necessary soil test should be carried out. Preparation of ponds during infection to eliminate the pathogens and preparation between the culture periods to further carryover of pathogens is also very important. When chlorine is used to treat the pond water, effective concentration should be used. Based on the research work carried out in CIBA, it was observed that effective concentration to kill WSSV only with water base is 5 ppm for 2 day, if the water contains infected animals (dead) it is 10 ppm 2 days, in soil based system, with planktonic WSSV, it is 15 ppm for 2 day and in soil based system, with dead infected animals it is 20 ppm for 2 days, Soil based system, WSSV (filtrate) added, water drained, exposed to sunlight for 2 days and then chlorine treatment, it is 10 ppm 2 days. Chlorine concentration also depends on the organic load and therefore should be determined based on the water condition. Accurate chlorine concentration will help to eliminate the pathogen, avoid unnecessary expenditure and will also help to maintain soil health condition. It is necessary to provide sufficient time gap between the culture practices. Again with respect to WSSV, the work carried out at CIBA indicates, the pathogen can remain viable for at least 35 days in non-drainable ponds and in drainable ponds it is 19 days. In addition to this other treatments such as drying the pond, ploughing and application of appropriate amount of lime should also be carried out.

Getting good water is an important aspect to carry out aquaculture practice. Water can be a primary source for the entry of pathogens. Wherever possible, it is advisable to go for recirculatory systems and thereby any pathogen entry can be avoided/minimised. Otherwise, it is necessary to go for adequate amounts of reservoir ponds where water can be stored initially, treated and finally matured before taking into culture ponds. Throughout the culture period, it is required to maintain good water quality and thereby avoid stress. Along with feed and other management practices, the aquatic environment should always be maintained healthy. Animals should be supplied with good quality diets and preferably supplemented with immunostimulants to maintain good immunity during the culture period and thereby avoid infection by opportunistic pathogens. Some of the commercial feed and live feeds can also serve as a source for pathogens. These feed should be properly tested before use. If necessary proper steps such as pasteurization should be adopted All the animals and their environment should periodically be monitored for their health status.

3. Pathogen management

Utmost care should be taken to pathogen management by preventing the entry. However, if due to unavoidable reason, if the pathogens enter the system, then take necessary steps either to eliminate it completely or reduce the number substantially to prevent mortality.

It is necessary to know the nature and virulence status of each pathogen and act accordingly. Many of the pathogens have reservoirs either as living organisms, water or inanimate objects. Therefore, one should be thorough with the nature of the pathogen and accordingly steps should be taken. Suitable environment and conditions for pathogen multiplication should be avoided. Ponds should have proper fencing system to avoid reservoirs or passive carriers for disease spread. It is always better to have preventive methods such as vaccination or use of immunostimulants than treatment. Similarly biological controls such as phage therapy should be preferred. However, during inevitable infection period, appropriate sanitizers should be used to reduce the pathogen load. Active ingredients and the mode of action of each chemicals should be known properly and accurate dosages should be used. Indiscriminate use of antimicrobials should be avoided to prevent stress on the animal and on the environment. It is necessary to maintain good diversity of planktons and bacteria in the pond. This will avoid the multiplication of pathogens. Biodiversity can be maintained through the use of good quality probiotics bacteria. Similarly, it is necessary to maintain proper plankton density in the pond and avoid bloom conditions.

4. People management

For all the people associated with either hatchery or farm, all the necessary biosecurity protocols should be put in place. However, this will be successful only when the people involved understand it clearly and practice it effectively. This involves management staff, workers and visitors those get a direct access to the aquaculture facility. Effective measures are required to prevent the entry of pathogens or spread through these people. Sensitive areas should be designated only for the authorised personnel through strict security arrangement.

Visitors from another farm are considered as serious risk factors and should be allowed after thorough sanitizing protocols. Disinfectant foot baths, hand washing stations or spray bottles, net disinfection station, vehicle disinfection station and showers should be in proper places to avoid pathogen entry. Sufficient and continuous awareness programmes should be arranged for all the employees to make them understand the basic principles and importance of biosecurity.

5. Aquaculture Quarantine Measures

Aquatic quarantine principle is applied to a cultured species coming to one area from the other area (within the country or another country). This is an important animal management and biosecurity measure. Through this procedure individual animals or population can be isolated and acclimatized to the new place for a specific period of time. During this period, these are observed for any abnormality or disease appearance. Even if these animals look healthy, these are tested for all possible known pathogens. If necessary, these are treated to make disease free. Once it is made sure that animals are free from pathogens, these are then either released to culture facility or live market. However, for exotic pathogens or presence of viral pathogens wherever treatment is not possible, the stock should be destroyed carefully making sure that the pathogen does not spread. The quarantine facility should be well designed and located in an appropriate isolated place or ensure that the facility is physically separated from farms/hatcheries. The facility should have easy access for transportation of animals, get sufficient quality water and the discharge water should be handled properly. The facility should have well established protocols and well trained staffs. This should have competent and readily available diagnostic support. While animals are maintained in quarantine facility, these be sampled at the beginning, at the end and at point of disease appearance and all the necessary tests should be carried out. Usually random and non-lethal sampling is required to be done to determine the health status. In case of detection of any disease, the entire stock should be properly destroyed/disposed.

India established its first state of the art AQF in the year 2009 at Chennai to facilitate *P. vannamei* broodstock import in India. However, India needs to establish separate AQFs for quarantine needs of various aquatic species such as ornamental fish, candidate finfishes such as seabass, corals, being proposed by the private sector for import. These facilities can be created under PPP mode, where central agencies, state Govt., research institutions and the private players can play a harmonised role. The import risk analysis (IRA) of CIBA clearly revealed that the aquatic quarantine at the importers‟ facilities was highly risky and recommended low-risk options to the Govt. of India to i) establish quarantine facilities under public sector with restriction on the culture practices and ii) establishment of SPF broodstock multiplication centre cum quarantine under public-private partnership with restricted permits for culture, as is presently being done with Pacific white shrimp. At large, the Quarantine and Biosecurity must be vested with the Govt agencies and Institutions as done in all other developed nations for effectiveness and delivery as per the regulations of the country.

6. Conclusion

Aquaculture sustainability is the need of the time and effective measures should be put in place to achieve the same. Both biosecurity and quarantine measures are essential parts of a healthy aquaculture practice and avoid disease appearance. With the adoption of new culture practices involving high stoking density and with the import of several new species for aquaculture, it is expected to have more disease prevalence and culture loss in the future. Therefore, these two systems are very much important and should be well understood and established to avoid disease occurrence, mortality and thereby huge economic loss. This will also avoid unwanted situations due to introduction of exotic pathogens or appearance of emerging diseases. Therefore, both the practices should accurately and appropriately be followed.

Bibliography

1. Arthur, J. R. (2004). The role of quarantine in preventing the spread of serious pathogens of aquatic animals in South-east Asia. In C. R. Lavilla-Pitogo & K. Nagasawa (Eds.), Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training. Proceedings of the Meeting on Current Status of Transboundary Fish Diseases in Southeast Asia: Occurence, Surveillance, Research and Training, Manila, Philippines, 23-24 June 2004 (pp. 25-33). Tigbauan, Iloilo, Philippines: SEAFDEC Aquaculture Department
2. Yanong, R.P.E. and Erlacher-Reid, C. (2012). Biosecurity in aquaculture, Part I: An overview. SRAC publication No.4707. Accessed online through http://fisheries.tamu.edu /files/2013/09/SRAC-Publication-No.-4707-Biosecurity-in-Aquaculture-Part-1-An-Overview.pdf on February 27, 2022.

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Water quality requirements and management for shrimp aquaculture

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Successful aquaculture largely depends on providing cultured animals with a healthy environment to grow. Among the various factors affecting aquaculture, water and soil quality are of paramount importance. Water quality variables affecting animal survival and growth are determining factors for disease outbreaks. Poor water chemistry leads to deterioration of water quality, which causes stress to the organisms being raised. Water quality variables such as salinity and temperature are important when assessing the suitability of a site for a culture of particular species. Dissolved oxygen, carbon dioxide, ammonia and other potential stressors play a major role during grow out period.

1. Physico-chemical characteristics

1.1 pH

Water pH is one of the most critical chemical parameters affecting aquaculture. It is determined by interactions among dissolved CO_2, carbonic acid, bicarbonate, carbonate and carbonate containing minerals. The pH decreases at night because of respiration and production of CO_2 by all organisms. The pH in ponds will rise during the day as phytoplankton and other aquatic plants remove CO_2 from the water during photosynthesis. Waters of moderate alkalinity are more buffered and the degree of pH fluctuation is lower than low alkaline water. The optimum level of pH for culture is between 7.5 and 8.5. For the best water quality, the maximum diurnal pH fluctuation should not exceed 0.5. Dolomite could be used at the rate of 180-300 kg ha^{-1} for 2-3 days to increase the pH when the pH difference in the afternoon exceeds 0.3. When the pH of the water exceeds the optimum level, it could be decreased by water exchange and use of fermented extracts.

1.2 Salinity

Salinity is the total concentration of all dissolved salts in water. Salinity determines osmotic relationships and also the growth and reproduction of the animal as well as its general metabolism. Salinity range of 10 to 35 ppt with variations not exceeding 5 ppt is considered as optimum level for growth and proper metabolic processes of culture species and also helps in reducing stress on the animal.

1.3 Temperature

Temperature is one of the factors controlling the rate of biochemical reactions and regulating the activities of cultured animals. The temperature below and above the optimum range (28 to 32^{o}C) is known to weaken the immune status of the animal making it more susceptible to diseases. The variation in temperature is known to lower the levels of total haemocyte counts, phenol oxidase and respiratory burst in addition to reduction in the activity of superoxide dismutase (SOD) responsible for scavenging superoxide anion. Increase in temperature favours high rate of evaporation which increases the water salinity beyond the tolerance level. Similarly, during the winter season, the low temperature will have a chilling effect reducing metabolic and growth rates of cultured animals.

1.4 Turbidity

Turbidity is an optical property of water which describes the cloudiness or muddiness of water. Turbidity of water arises from both biotic and abiotic factors such as plankton, dissolved organic substances, and suspended sediment such as silt or clay and solid particles. Turbidity due to plankton is desirable but turbidity arising from soil particles is undesirable. Turbidity can be measured in terms of transparency using Secchi disk. The optimum range of transparency is 25–50 cm. High value of transparency (> 60 cm) is indicative of poor plankton density and low value (< 20 cm) indicates high density of plankton. High plankton density may affect fluctuations in dissolved oxygen and pH in the pond. Clay turbidity reduces the light penetration and thereby affects the photosynthesis and natural productivity of the ponds. High turbidity may cause temperature and dissolved oxygen stratification in aquaculture ponds.

1.5 Total suspended solids (TSS)

TSS is particles that are larger than 2 microns found in the water column. Anything smaller than 2 microns (average filter size) is considered a dissolved solid. Most suspended solids are made up of inorganic materials, though bacteria and algae can also contribute to the total solids concentration. These solids include anything drifting or floating in the water, from sediment, silt, and sand to plankton and algae. Organic particles from decomposing materials can also contribute to the TSS concentration.

2.

3. Chemical characteristics

2.1 Dissolved oxygen (DO)

Dissolved oxygen refers to non-compound oxygen or free oxygen (O_2) that is not bonded to any other element. It is the most important water quality variable in aquaculture, and it is expressed as parts per million (ppm) or as a percent of saturation value for that temperature and pressure. In ponds, there are three primary sources of oxygen, direct diffusion from the atmosphere, wind and wave action, and photosynthesis. A healthy phytoplankton bloom is essential to keep dissolved oxygen concentrations within a safe range. Algal bloom crash due to rainy weather and lack of essential nutrients leads to restriction of photosynthesis and therefore, limiting the availability of oxygen. The concentration of toxic substances such as unionized NH_3, hydrogen sulphide, and carbon metabolites such as methane increases when a low dissolved oxygen level exists. However, in the presence of an optimum level of oxygen (4-10 ppm), the toxic substances are converted into their oxidized and less harmful forms.

2.2 Total alkalinity

Alkalinity is the water's ability to neutralize acid without changing the pH and is a measure of the total concentration of bases (bicarbonates, carbonates, phosphates, hydroxides) in pond water predominantly bicarbonate and carbonate. Waters with high alkalinities generally have a greater complement of most ions than water of low alkalinity; it also increases pH that favours rapid decomposition of organic matter by organisms. In addition to that phosphorus availability will be increased by increasing total alkalinity by the addition of lime, which helps for phytoplankton growth. To improve alkalinity and stabilize pond water quality, dolomite, shell lime, calcium carbonate, egg shells and zeolite could be added depending upon soil pH and buffering capacity. If alkalinity is low, lime or dolomite should be applied @ 180-300 kg ha^{-1} for every 2-3 days' interval at night until the pH reaches the required levels. Agricultural limestone will not increase pH beyond a maximum of 8.3. The use of hydrated lime [$Ca(OH)_2$] or quick lime (CaO) is not recommended because either of these compounds can cause the pH to rise very rapidly to levels that are harmful to aquatic life. Sodium bicarbonate is an alternative as it dissolves quickly compared to the limited solubility of traditional liming materials. To reduce the alkalinity level, EDTA @ 20-30 kg/ha can be applied at night.

2.3 Total hardness

Total hardness is the sum of the concentrations of calcium (Ca^{2+}) and magnesium (Mg^{2+}) in water, expressed as mg L^{-1} equivalent $CaCO_3$. It is a general indicator of the degree of mineralization of water, and as total hardness increases, concentrations of most other substances also tend to increase. The nature of water supply largely determines the hardness of pond water. The level of total hardness is strongly correlated with total alkalinity because of their frequent common origin from the dissolution of limestones. Hence, hard waters often have moderate to high total alkalinities, are well buffered at a neutral to slightly alkaline pH, and tend to be more productive than poorly mineralized, acidic waters. A low level of hardness can cause stress to animals and high level is not desirable as it increases the pH and reduces the availability of other nutrients.

2.4 Minerals

Minerals are important for the growth and metabolism of animals. Calcium and magnesium are essential for the biological processes of fish-bone and scale formation, blood clotting and other metabolic reactions. Calcium is important in the moulting process of shrimp and other crustaceans and plays a major role in hardening of the shell. Crustaceans absorb calcium from the water when moulting, and if the water is too soft their exoskeletons begin to soften and they may cease to moult. Calcium reduces the toxicity of metals, ammonia, and the hydrogen ion. Major ion deficiencies can have serious physiological consequences ranging from stunted or poor growth through to asphyxiation, oedema and death. Potassium has an essential role in regulating sodium and therefore fluid balance within the haemolymph. Hence, there is a need to supplement potassium as and when required.

The primary candidate of choice for shrimp culture in low salinity water (LSW) is the Pacific white shrimp, *Penaeus* (*Litopenaeus*) vannamei in most parts of the world due to its remarkable ability to effectively survive and grow at extreme salinities. The sources of LSW can vary greatly such as, low salinity aquifers/ brackish water derived from estuaries diluted with freshwater /high saline brine solution mixed with freshwater/ river waters in coastal inland areas. Irrespective of the source, there is a huge amount of variation in both the salinity and ionic profiles of LSW sources compared to seawater diluted to the same salinity. The ionic composition of water is more important than the salinity. Despite the success of rearing shrimp in LSW by many farmers in India, variable growth and survival among ponds are still being reported. Physico-chemical analysis of LSWs at different geographical locations in Andhra Pradesh (AP), Tamil Nadu (TN) and Gujarat by CIBA revealed that all saline well waters are not equal, even if they originate from the same aquifer. Researchers reported that these waters tend to be low in potassium (K), magnesium (Mg) and sulfate (SO_4) relative to the concentrations expected in seawater diluted to the same salinity. It was reported that although high levels of Ca seem to be necessary, the ratios of ions Ca:K, Ca:Mg etc. are also important. Aquatic organisms can absorb calcium and magnesium directly from the water or from food. The presence of free calcium at relatively high concentrations in culture water helps reduce the loss of other salts (e.g. sodium and potassium) from body fluids. Sodium and potassium are the most important salts in hemolymph and are critical for normal heart, nerve and muscle function. In low calcium water, fish can lose substantial quantities of these salts into the water. The ratio of Na to K and Ca to Mg in the water are highly important for survival, growth and production rather than salinity. The ratio of minerals should be maintained similar to the ratio of sea water.

Table 1. Ionic composition of Seawater, Brackishwater and Freshwater

Ions	**Seawater**	**Brackishwater**	**Freshwater**
Ionic composition (mg/l)			
Chlorides	19000	12090	6
Sodium	10500	7745	8
Sulphate	2700	995	16
Magnesium	1350	125	11
Calcium	400	308	42
Potassium	380	75	2
Bicarbonate	142	156	174
Other	86	35	4
Total	34558	21529	263
Ionic ratios			
Ca/Mg ratio	0.3	2.46	3.82
Na/K ratio	28	103.3	4.0
Ca/K ratio	1.05	4.11	21.0

Recently farmers are culturing vannamei over varying salinity from 0-40 ppt. The ionic ratio varies with the salinity of the water. In order to maintain the optimum concentration of minerals and ionic balance, modifications in mineral supplementation through water and diets are available. Water modification approaches are more effective compared to the dietary modification strategies though the cost of ionic fortification is comparatively high when the culture area is large. Ionic levels in the low saline water ponds have to be raised to their concentration in seawater diluted to the same salinity of production ponds.

Table 2. Desired mineral levels at different salinities

Mineral	**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**

The amount of salt to be added in the pond will be calculated based on the desired mineral level and the selected salt. Due to lack of awareness about minerals supplementation, farmers are applying higher amount of mineral mixtures to the ponds. An actual requirement is difficult to quantify due to the variability in the ionic profiles of pond waters. No pond water will be exactly the same and the bioavailability of minerals will be a function of their concentration in the water. The Institute analysed the efficiency of commercial mineral mixtures in terms of availability of minerals at different salinities.

- Dose of the product to get a desired concentration of a particular mineral (g/m^3) = Desired concentration of particular mineral (mg/L) ÷ Percentage of that particular mineral in salt/100.
- For example, if you want to use magnesium sulphate (Epsum salt) containing 10% Mg to increase Mg concentration by 25 mg/L: Dose of salt = 25 ÷ 10% /100 = 250 mg/L.

Generally, under high or low saline waters with optimum minerals concentration and proper ionic ratios, there is no need of supplementation. However, throughout the culture period, major minerals are lost due to soil adsorption, shrimp harvest, draining at harvest and seepage, altering their concentration. Hence, there is a need to evaluate the mineral concentrations in pond waters regularly (twice weekly) and to supplement them in case of any deficiency. Balanced mineral profiles are to be maintained to encourage proper molting and healthy growth.

4. Metabolites and management

The major metabolites in aquaculture are ammonia, nitrite, and sulphide. Feed and fertilizers contribute a large proportion of nitrogen, which is necessary for growth or maintenance of aquatic animal is catabolized into ammonia and eliminated by diffusion through the gills into the water. Nitrite is the intermediate product of the oxidation of ammonia to nitrate. In the absence of sufficient level of nitrite oxidizing bacteria, nitrite level will increase in the water. Hydrogen sulphide is an odiferous, flammable gas, highly toxic to aquatic animals. It is mainly produced as sulphur reducing bacteria decompose organic matter under anaerobic conditions

Total ammonia concentrations are generally higher in ponds receiving large amounts of feed and at the time of reduction of ammonia assimilation by phytoplankton. The fraction of ammonia depends on pH, temperature, and to a lesser extent on salinity. Un-ionized form is considered a more toxic form of ammonia due to its ability to diffuse readily across the cell membrane. For a healthy pond environment, unionized ammonia level should be less than 0.1 mg L^{-1}. Nitrite (NO_2) is the intermediate product of bacteria mediated conversion of ammonia to nitrate. Imbalance in levels of denitrifying and nitrifying bacteria leads to accumulation of nitrite. Among the metabolic toxicants, nitrite is considered most dangerous as it can accumulate in haemolymph up to 10 fold higher than in water via active chloride uptake mechanism and passive entry. Nitrite is more toxic in low saline conditions compared to brackish and seawater based culture ponds.

Under anaerobic condition, certain heterotrophic bacteria can use sulphate and other oxidized sulphur compounds as terminal electron acceptors in metabolism and excrete sulphide. It is an ionization product of hydrogen sulphide and pH regulates the distribution of total sulphide among its forms (H_2S, HS- and S^{2-}). Un-ionized hydrogen sulphide is toxic to aquatic organisms. Concentration of 0.01 to 0.03 mg/l of H_2S may be lethal to aquatic organisms and any detectable concentration is undesirable.

The use of aerators results in the mixing of water at surface and bottom and breaks down dissolved oxygen stratification and also can eliminate black mud formed at the interface of pond water and bottom mud. Paddlewheel aerators are commonly used, which is capable of elevating the dissolved oxygen level from 0.05 to 4.9 mg L^{-1} within 4 hours in a half-hectare pond. Generally, one horsepower of aerator is suggested for every 500 kg of biomass. Another alternative is to use approximately 500 ml of H_2O_2 ha^{-1} to increase DO concentration by 1.5 ppm.

Table 3. Optimum water quality parameters for aquaculture

Parameter	Optimum range
Temperature (^oC)	28 - 32
pH	7 .5 - 8.5
Salinity (ppt)	15 – 25
Transparency (cm)	30 – 40
Total suspended solids (ppm)	<100
Dissolved oxygen (ppm)	>4
Chemical oxygen demand (ppm)	<70
Biochemical oxygen demand (ppm)	<10
Total alkalinity (ppm as $CaCO_3$)	120-200
Total ammonia N (ppm)	< 1
Unionised ammonia N (ppm)	<0.1
Nitrite N (ppm)	<0.25
H_2S (ppm)	<0.003
Nitrate N (ppm)	0.2 - 0.5
Phosphate (ppm)	0.1 - 0.2

3.1 Bioremediation

Microorganisms are of major importance in aquaculture, as they are present in the water, sediments and as well as in and on the aquatic animals like fish and shrimps. Microorganisms have special impact, especially on the aquatic zone of ecosystem where light cannot approach. Some microbes are decomposers which have ability to recycle the nutrients. They also play a positive role in the elimination of toxic wastes in the aquatic environment like ammonia, nitrite and hydrogen sulphide. Hence, they are also used for microbial bioremediation of aquatic environments. Biological equilibrium between beneficial and detrimental microorganisms in the aquatic environment has direct impact on the health status of aquatic animals. These and other functions make micro-organisms key players in the health and sustainability of aquaculture. Dissolved and suspended organic matter mainly carbon chains, carbonaceous wastes, leached or excess feed, feces, non-organic particulate form. Bacteria will mobilize C, N, P. Bacteria used for the bioremediation of organic detritus are *Bacillus subtilis, Bacillus licheniformis, Bacillus cereus* and *Bacillus coagulans.*

5. Shrimp farm discharge water management

Waste production levels from extensive and semi-intensive systems of shrimp culture are low compared to intensive culture systems. The waste from shrimp culture ponds contain mainly suspended solids, comprising unconsumed feed, faecal matter and plankton, dissolved nutrients phosphorus and nitrogen and metabolites such as ammonia and nitrite. Presently, many farms lack effluent treatment systems or discharge water treatment systems (DWTS) for treating the discharge water before it is released into the open waters. Coastal Aquaculture Authority (CAA) in its guidelines for sustainable development and management of brackishwater aquaculture has prescribed standards for the water discharged from the shrimp farms. The establishment of cost-effective DWTS is necessary to bring the shrimp farm discharge water within the prescribed standards and mitigate any adverse impact on the ecology of the open waters.

In conclusion, water quality parameters play an important part in maintaining pond health to achieve higher production, productivity in a sustainable way. Aquaculture farmers need to constantly monitor pond water health and undertake timely and appropriate interventions for maximizing economic benefits. Inland saline waters are deficient in potassium and rich in calcium, results in imbalanced ionic ratio. To rectify the skewed ratio, minerals should be applied externally.

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Soil quality requirements and management for shrimp aquaculture

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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. The soils are classified mainly under eight major heads: alluvial, black, red, laterite, forests, desert, saline and alkaline and peat in India. Generally acidic soil and acid sulphate can cause low pH and high sulphide production respectively, unless proper management practices followed, these soils are not suitable for aquaculture.

1. Soil requirements

In general, the soil requirements for shrimp aquaculture are given in Table 1.

Table 1. Soil requirements for shrimp aquaculture

Parameter	Optimum range
pH	6.5-7.5
Organic C (%)	1.5-2.0
$CaCO_3$ (%)	>5.0
Av. N (mg/100g)	50-70
Av. P (mg/100g)	4-6
EC (mmhos/ cm)	>4

1.1 Soil type

The brackishwater aquaculture is done on salt affected soils or coastal soils. Saline soils are classified as saline if the EC exceeds 4 or more dS/m at 25^0C, exchangeable sodium < 15% and pH < 8.5. Alkali soils have high sodium content causing dispersion of organic matter. The solution extracted from the saturation paste have an EC > 4 dS/m at 25^0C, exchangeable sodium >15 % and pH between 8.5 and 10.0.

1.2 Soil texture

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. The clayey soils rich in organic matter promote growth of benthic blue algae, which along with other micro-organisms constitute the main food of brackishwater animals. 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. Moderately heavy textured soils are suitable for pond preparation. Hence, some of the textures suitable for aquaculture are- sandy clay, sandy clay loam, clay loam.

1.3 Soil pH

The pH indicates whether the soil is acidic or alkaline and is an important parameter which affects pond condition. Slightly acidic to slightly alkaline soil pH is suitable for higher production. The nutrient availability, mineralization rate, bacterial activities and phosphorus fixation are influenced by pH. The 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.

1.4 Organic matter

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.

1.5 Calcium carbonate

This parameter gives an indication of the amount of free $CaCO_3$ present, the absence of which shows acidic reaction. The harmful effects of sulphides and acids can be reduced by application of lime which is calcium carbonate. The soils with high calcium carbonate content promote biological activity and hence accelerates breakdown of organic matter. This creates more oxygen and C reserves in the soil. The $CaCO_3$ precipitates suspended or soluble organic materials, decreases BOD and increases nitrification due to requirement of Ca by nitrifying microbes. A productive soil should have $CaCO_3$ more than 5%.

1.6 Soil salinity

Saline soils are potentially productive soils. The excess of Na ions in these soils exerts antagonistic effects on Ca and Mg absorption. These soils commonly occur in arid and semi-arid regions nearer to the sea and the salinity increases with the increase in salinity of water.

2. Pond preparation

Good bottom soil and water quality are vital ingredient for any successful aquaculture practices. Good pond management is critical as the water quality can deteriorate quickly due to the accumulation of organic matter from uneaten feed, faeces, dead shrimp and algal bloom crashes. 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.

2.1 Pond preparation after harvest

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

2.2 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. After draining, pond should be desilted. In wet method, after the final drain harvest, the accumulated black material on the pond bottom is flushed in the form of thin slurry using a high pressure pump. The advantage of this method is that waste is removed in suspension. This method needs a settling pond where waste is removed from the water and treated repeatedly to avoid polluting the local environment.

2.3 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. After drying, the waste can either be removed manually or with machines. Pond drying between crops is a common practice for releasing nutrients to the pond water in brackishwater aquaculture ponds. In aerobic decomposition, the organic matter is oxidized to inorganic substances such as carbon dioxide, water, ammonia, sulphate, phosphate etc. Drying and cracking of pond bottom enhances aeration and favours microbial decomposition of soil organic matter. The optimum moisture content for drying is 20%, but it might vary among soils from different ponds. To enhance the oxidation of wet patches nitrate salts at 20-40 g/ m^2 could be applied. The nitrate salts enhance the organic matter degradation by acting as nitrogen for microbes.

Properly dried pond bottom

Unevenly dried pond bottom

(Source: Boyd, et al., .2002)

2.4 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 practice lime materials such as agricultural limestone ($CaCO_3$, quick lime or unslaked lime (CaO), and hydrated lime or slaked lime [$Ca(OH)_2$] are commonly used in agriculture to increase pH of bottom soil, accelerating the microbial activity.

The lime requirement of a soil can be defined as the amount of lime material that must be added to raise the soil pH to 7.0. First, the amount of lime needed as pure calcium carbonate is calculated based on the actual pH of pond soil and the extent of the area to be applied. The recommended dose for various lime materials was calculated by dividing the value of lime needed as pure $CaCO_3$ with the percent effective calcium carbonate (PECC) value of that particular lime material. Liming can be done by broadcast over dried pond which includes the dike inner walls and by mixing with water and spraying over the pond bottom.

Table 2. 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	1 1.5 to 9.2
4.0 to 4.5	17.0 to 14.2	16.6 to 13.9	13.8 to 11.5

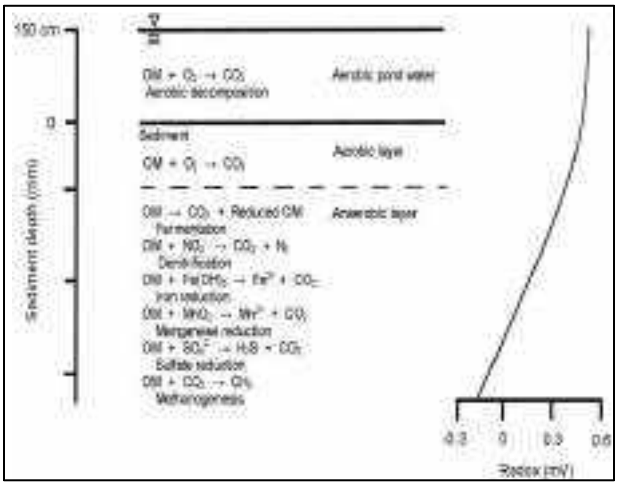
3. Management of pond bottom during culture period

All aquaculture pond bottoms become covered with sediment, and this sediment can be considered as aquaculture pond soil. In describing various physical, chemical and biological processes occurring in the pond bottom, it is convenient to refer to bottom deposit as sediment. he oxidized layer at the sediment surface is highly beneficial and should be maintained throughout the shrimp culture. Metabolic products of aerobic decomposition are carbon dioxide, water, ammonia, and other nutrients. In anaerobic sediment, some microorganisms decompose organic matter by fermentation reactions that produce alcohols, ketones, aldehydes, and other organic compounds as metabolites. Other anaerobic microorganisms are able to use oxygen from nitrate, nitrite, iron and manganese oxides, sulphate, and carbon dioxide to decompose organic matter, but they release nitrogen gas, ammonia, ferrous iron, manganous manganese, hydrogen sulphide, and methane as metabolites. Some of these metabolites, and especially hydrogen sulfide, nitrite, and certain organic compounds, can enter the water and be potentially toxic to shrimp. Methane and nitrogen gas pass through the layer and diffuse from the pond water to the atmosphere. These two gases do not cause toxicity to aquatic organisms under normal circumstances.

3.1 Monitoring of soil parameters during culture period

Monitoring of soil quality condition can be valuable in shrimp culture pond management. During culture the carbonaceous matter, suspended solids, faecal matter and dead plankton etc. also settle at the pond bottom. Major concerns in pond bottom soil management are low soil pH, high soil organic matter, loss of the oxidized layer, and accumulation of soft sediment. Pond managers should still strive to prevent severe soil quality problems from developing. In older ponds with impaired soil quality, problems should be corrected and prevented from recurring. These materials have combined effect on the environment of the pond bottom. In order to understand the pond botton condition, a pond core sampler fabricated by the Environment Group of CIBA can be used for the depth-wise collection of cores. To understand the condition of the pond bottom, the parameters to be monitored regularly are: pH, organic carbon content and redox potential. 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 system in the pond may also help in the removal of organic waste periodically.
- Water circulation by water exchange, wind or aeration helps to move water across mud surface and prevent the development of reduced condition. Bottom should be smoothened and sloped to facilitate draining of organic waste and toxic substances.
- Bottom Raking - The oxygenated water and surface should be always in contact for the purpose of maintaining the oxidized layer. Stirring the bottom layer by manual raking and chain dragging are the common methods to improve the contact with oxygenated water maintain the oxidized layer.



Reactions at the pond bottom soil during aerobic and anaerobic conditions

On-farm measurement of redox redox potential

ORP can be measured at soil water interface (SWI) near sluice gate and away from the aerators by portable multi parameter analyser with ORP probe. If probes are not available, the sediment sample at 10-cm depth is to be collected in a polythene bag under air tight condition near sluice gate and away from the aerators. Once the sample is brought out of the pond, immediately ORP has to be measured under air tight condition by using a portable/ bench top redox meter. In order to minimise the errors of ORP variability, minimum of three sampling places have to be fixed near sluice gate and repeated measurements are to be taken at each sampling place (SWI or 10-cm depth soil in polythene bag) and the average value can be taken as final value.

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Significance of feed in sustainable aquaculture

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Indian aquaculture sector with a share more than 4.3 MMT of aquaculture produce which is consumed internally, while production of farmed shrimp in India crossed 0.43 MMT mark in 2014-15, which constitute almost 70% of annual seafood exports from India, with a value of about $5 billion. Brackishwater aquaculture is poised for substantial growth in India in the coming years and is being considered as one of the economic engine which can generate food, employment and profit. Successful aqua farming is dependent on availability of high quality balanced feeds which gives best growth and health with minimal waste output at the lowest possible cost. In this article the current status of shrimp feed sector and the way forward towards sustainable brackishwater aquaculture development, with special reference to Indian shrimp aquaculture sector are discussed.

History of shrimp farming in India

In India, shrimp culture has been carried out as a traditional activity since ages. For a developing country like ours, shrimp farming is a high potential sector with enormous scope for increasing the foreign exchange and employment generation. Early nineties witnessed a phenomenal growth of the sector which was entirely dependent on the culture of a single species, the tiger shrimp, *Penaeus monodon*. During this period, the shrimp culture was a low-risk, high-profit venture. In late 90s, there were serious problems of viral diseases and environmental safety issues, which arose mainly because of the lack of planning and regulation. The Central Institute of Brackishwater Aquaculture (CIBA) and National Bureau of Fish Genetics and Resources (NBFGR) with the aim of evaluating the feasibility of the introduction of this new species and carried out the risk analysis for introducing the exotic shrimp, *Penaeus vannamei.* During 2009 the Coastal Aquaculture Authority (CAA) under the ministry of Agriculture, approved vannamei culture through import of Specific Pathogen Free (SPF) brood stock, stipulating policy directives with strict regulatory guidelines. Since the introduction of vannamei, Indian shrimp farming has witnessed the rapid shift towards the culture of vannamei almost replacing tiger shrimp, except the tradition culture practiced in West Bengal and Kerala, where still monodon and indicus are farmed. Inorder to sustain the rapid growth of vannamei farming, feed industry also need to support the sector with quality and cost-effective feeds, as feed alone contributes for 50-60% of the operative cost in the semi intensive shrimp culture practiced by Indian farmers.

Genesis of shrimp feed Industry in India

Feeding of shrimp in the traditional system of culture has been mainly with farm made feeds using locally available ingredients till 1990 and with the advent of improved traditional and semi intensive system the necessity for quality formulated feed assumed significant importance in the country and the requirement was met by importing feeds from Taiwan and Thailand. This has been gradually replaced by feeds produced by feed mill operating domestically, mostly as joint venture with the overseas players. These feed mills were either established by foreign multinational players or Indian firm with foreign technology support, started producing the manufactured shrimp feed. Even now majority of shrimp feed produced in the country have collaboration with multinationals or foreign consultants. However, there are small and medium scale indigenous feed mill also in operation and catering to the need of own use or job work to meet the requirement of preparing feeds on request by the farmers using locally available ingredients. In this context ICAR- CIBA has developed cost effective formulated feed for shrimp, the 'Vannamei ^{Plus}' and the technology transferred to the entrepreneurs, big farmers on a non-exclusive basis, and this model has become a success and gaining popularity, where already 4 feed mills has come up in the states of AP and Gujarat.

Shrimp feed scenario

Feed being a major input in the farmed aquatic animal production, India's industrially produced feed has grown to the tune of 1 to 1.25 MMT in year 2014, in which the share of shrimp feed alone is 0.6 MMT. After the introduction of *Penaeus vannamei* during 2009 , the shrimp feed business in the country underwent a sea change. Existing feed companies expanded their production and operation capacity and new players entered the feed business. The total installed capacity of feed mills for shrimp feed production in India is about 1.6 MMT and about 0.75 MMT of shrimp feed was produced during the year 2016. Currently there are about 40 organized aqua feed manufacturing plants (Table-1) with installed production capacity of around 2 to 2.3 MMT per annum, and it continues to expand further. Most of the feed mills are concentrated in AP followed by Tamil Nadu. Recently new feed mills are being established in the states of West Bengal, Odisha, Gujarat and Kerala. It is apparent that the installed feed production capacity of the shrimp feed mills are more than sufficient to meet the growing demand. The information on state wise shrimp feed consumption pattern (Fig 1) revealed that AP is the leading state almost accounting for two thirds of total feed produced followed by Gujarat, Odisha, Tamil Nadu and West Bengal. The average seasonal consumption of shrimp feed in different months are given in Fig 2. Here it is pertinent to note that the feed demand is seasonal. There are two main crops in India viz., summer crop and winter crop. About 60% of the total feed produced is consumed during the period from April to July indicating the peak season for shrimp feed consumption during the summer crop.

Table- 1. List of Major Shrimp feed producers

S. No.	Name of the feed company	S. No.	Name of the feed company
1	Charoen Pokphand India pvt. Ltd.,	16	Sai Aqua feeds
2	Avanti Feeds	17	Sonac
3	Godrej Agrovet	18	Bismi Feeds
4	Growel Feeds	19	Coastal feeds
5	The Waterbase	20	Deepak Nexgen
6	Grobest Feeds Corporation (India) Ltd.,	21	Matrix Seafood India Pvt Ltd.,,
7	Nexus Feeds	22	Ananda Aqua Exports
8	Fedora seafoods private Limited	23	Uno Feeds
9	Devi Feeds Pvt Ltd.,	24	RNK Agro and Chemical Limited
10	Shenglong	25	Golden feeds ltd
11	Cargill India Pvt Ltd.,	26	Anmol Feeds Private Limited
12	Sharat Industries Limitted	27	Kingmei Feeds
13	IB Abis Shrimp Feed	28	NG Feeds Pvt Ltd.,
14	Unibait feed Pvt Ltd.,	29	PVS group limited
15	BMR feeds	30	Devee biologicals pvt ltd

- In addition to the above there are ten small scale feed mill catering to the need of self-requirement /doing job work

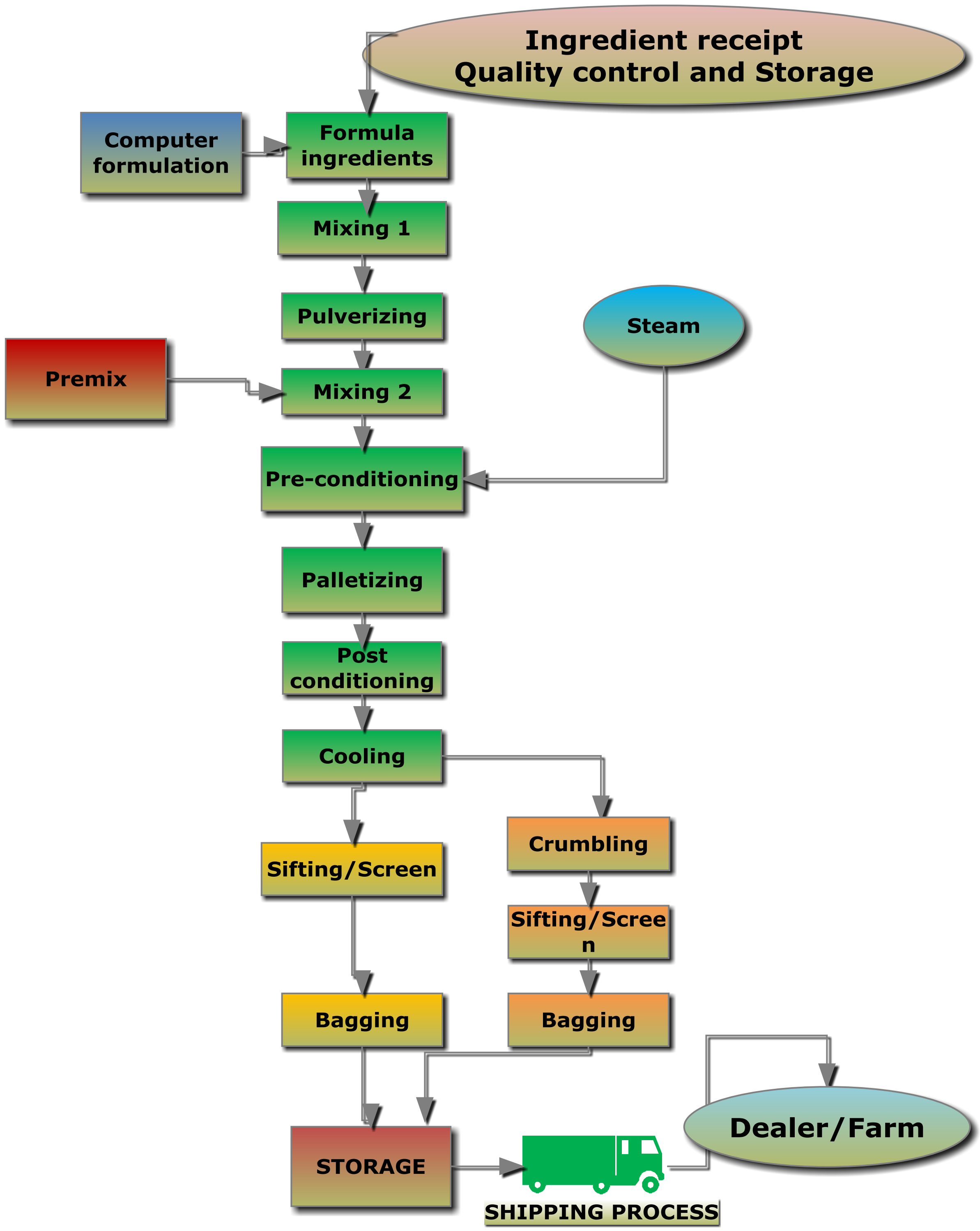
Under construction:

1. Nippai Shalimar Feeds Private Limited
2. Falcon Feeds
3. IFB Agro Industries

Most of the organized manufacturers of aqua feed have both pelletizing and extrusion capabilities for making shrimp feeds and fish feed respectively. Shrimp farming depends 100% on industrially manufactured formulated feeds. Increasing feed price is a major challenge facing Indian aquafeed industry, which is not only as a consequence of the increase in the ingredients cost such as fish meal, but also due to the increasing cost towards, energy, multi layered marketing network and logistics.

Feed processing

Most of the corporate and big feed mills are having the state of the art facilities for production of quality water stable shrimp feed pellets. The shrimp feed mills have facilities for coarse grinding, fine grinding, sieving, mixing and Steam pelleting with three stage pre conditioner (Figure 3) combined with post-pellet conditioning is in vogue. Most of the shrimp feed produced in India uses a ring die pellet mill to produce a compact pellet. In general the shrimp feeds for tiger shrimp consist of three grades of crumble (C1, C2 and C3) and three to four grades of pellets in the diameter of 1.8, 2.0 and 2.2mm. However there is a gradual shift in sizes of the pellet in the feed meant for pacific white shrimp, *Penaeus vannamei*. Now the feed pellet sizes are available as 1.2, 1.4, 1.6, 1.8 and 2.0 mm are being used for feeding vannamei. Though production of feed pellets at sizes less than 1.8mm is a high energy consumption process, feed miller and farmers are of the view that smaller pellets are better and could able to meet the requirement of current practise of white shrimp farming at higher densities than *P.monodon*. The inherent advantage of smaller diameter pellet is that, crumbling is a two-step process wherein bigger pellets (1.8mm) are subjected for crumbling to get the required size(400 micron to 1.7mm) and the time delay in crumbling operation is reduced in case of taking production with smaller size dies. Attempts have been made to produce extruded shrimp feeds with reasonable success and few firms stated producing the extruded shrimp feeds. The nutrient specification used by commercial shrimp feed manufacturers for vannamei are shown in Table -2



Flow chart for shrimp feed production

Table -2. Average nutrient composition of different grades of commercial shrimp feeds for vannamei

Feed code	Crude Protein % (Min)	Crude Fat% (Min)	Moisture% (Max)	Crude Fibre % (Max)
Crumble 1	35-36	5-6	11-12	3-4
Crumble 2	35-36	5-6	11-12	3-4
Crumble 3/ Pellet 1	35-36	5-6	11-12	3-4
Pellet 2	35-36	5-6	11-12	3-4
Pellet 3	35-36	5-6	11-12	3-4
Pellet 4	34-36	5-6	11-12	3-4
Pellet 5	34-36	5-6	11-12	3-4

In general the crude protein content of commercially available vannamei feed in India varied from 34 to 36% while the lipid content was in the range of 5-6%. Most of the firms have uniform nutrient content for different grades of grow out shrimp feed, while some have one or two per cent decreased protein content in the feed meant for last 30 days of culture. Indian shrimp farmers are keen on physical quality and they expect feeds to have at least two to three hours of water stability and the other criteria they look are consumption and FCR. The final quality would be assessed based on the final harvest details on growth, FCR and survival. Most of the feed companies declare the nutrient composition of feeds in the feed bag and the practice of testing the feed for its nutrient content by the farmer is not common. Bureau of Indian standard has developed standard for marine shrimp feed and this standard compliance is a voluntary one.

Organic shrimp feed, for raising organic shrimp

There is an increased interest and acceptance for certified organic seafood in global market. The seafood farmed in the organic aquaculture systems, which is based on minimal use of off-farm inputs and promotes the harmony between species and ecosystem, fetches a premium price in the market. The most challenging issue in the organic aquaculture is the availability of certified organic feed, in the market. In India this is an emerging concept and there is no organic shrimp feed in the market. Realising the importance of organic shrimp farming, ICAR-CIBA has developed an organic shrimp feed by adopting the organic standards, and got organic certified. To scale up the organic feed, CIBA has entered into an agreement with M/s. Jass Ventures Pvt. Ltd. for joint-development and transfer of organic shrimp feed for the sustainable farming organic tiger shrimp. Construction of feed mill to produce organic shrimp feed is under process.

Shrimp feed marketing

Most of the big and corporate feed mills have their own marketing network which is strong enough to sell their feeds through the network of distributors/dealers. Big farmers and corporate farms have the direct access to the feed mill and because of their sizeable requirement of feed they could able to buy directly from the feed mills for reasonable cost advantage. Feed mills also explore ways to increase feed sales through their technician and local consultants who are having direct contact with the farmers and could able to influence the decision making in purchasing the feed from a firm or a specified brand. Farmers are attracted by quality feeds, good feed conversion ratios, healthy shrimp and successful crops. The feed millers are selling the feed on cash and carry basis, While the dealers are marketing the feed at large on credit basis. Small and marginal farmers could purchase through credit only and this is one of the main factor responsible for higher cost of production of shrimp as the feed price is higher for credit and the interest is calculated from the date the first consignment of feed supplied to the farmer. One of the prudent strategies to reduce the feed cost is by by-passing the distribution network and providing opportunities for the farmer to buy the feed directly from the feed manufacturer. This can be achieved by establishing a small or medium scale feed mill in each 300 - 400 ha shrimp farming area, with production capacities of 1-2 tons/hr, with an investment of 1 to 2 crores . In this scenario there is option for sourcing and using locally available ingredients to reduce the cost and the extra cost associated with marketing and distribution are avoided as the feed milers would directly deliver to the farm site or the farmer would procure directly from the feed mill. Alternatively a group of farmers can also jointly establish a small scale feed mill under the farmers' cooperative and they themselves can produce to meet their own feed requirement.

Feeding and Feed Management

Feed management is the prime factor influencing the profitability in shrimp farming. 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 shrimp and production. There is a strong relationship between feed and stocking densities, hence feed management becomes important for managing the ponds more effectively. There are several factors influencing feed and feed management in commercial shrimp aquaculture.

Most of the feed millers provide feeding charts for feeding shrimp during the period of culture operation. These tables are based on either by some experiences or based on theoretical models. These are guideline only and the actual feed requirement will vary depending on the biological and environmental conditions of the pond. Since most of the feeding charts are based on size of shrimp and biomass in the culture pond still errors occur because accurate estimation of biomass in a pond is very often not possible correctly by all the farmers. 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**.**

In the first thirty days of culture blind feeding or the concept of full feed is being practiced by the farmer. This is carried out by based on the guidelines prescribed. To start with fixed quantity of feed is offered in relation to the stocking and the feed is increased incrementally upto thirty days of culture. Alternatively full feed for the Pl is advocated at 60-30% of the assumed body weight and it is gradually reduced to 10% at the end of 30 days. By this time the shrimp would have attained the average body weight of 3 gram. From 30 days onwards the feeding has been regulated based on the standing biomass and check tray observations. First thirty days the crumbled feed are broadcasted from the pond dykes along the entire length and width of the pond. If the air current is more, then it is advisable to wet the crumble and broadcast the feed. From 30 days onwards, boat feeding or float feeding (Figure 4) is advocated and this has to be carried out covering the entire water spread area. Normally four to five feeding is recommended. In India farmers expect very high ADG in the farming of shrimp and from 50 days onwards farmers are more keen about the daily weight gain and they expect a ADG of 0.25 to 0.3 gram up to 100 days of culture during which time the shrimp would have attained average body weight of 22 to 25 gram. The ADG shows a plateau or a slight decreasing trend after 100 days of culture. In the feeding of vannamei owing to their higher stocking densities the quantum feed offered per meal has increased considerably and the higher feed requirement in the later stages of farming poses problem in manual feeding. In order to deliver the feed uniformly and as per the requirement auto feeders are being used. Various types of auto feeders are available in India. Auto feeders are mainly used by big farmers and corporate shrimp farms wherein there is a crunch for manual labour. The main advantage in using the auto feeder is an appreciable improvement in FCR and improved soil and water quality compared to manual feeding. CIBA has also developed solar based cost effective auto feeder (Figure 5) and successfully demonstrated and this feeder is ready for commercialisation.

Feed ingredients

India being a major agriculture based economy, there are plenty of ingredient resources spread across vast stretches of the country in a patchy manner. It is one of the biggest producer of rice, wheat and oil seeds. These agricultural products and by products along with marine protein sources are the staple ingredients in shrimp feed. The principal classes of feed ingredients of Indian aquafeed basket can be classified as, fish meal and other marine protein sources, vegetable protein sources, cereals and its by-products.

Marine protein sources

Indian aqua feed sector judiciously manages the fishmeal trap to ensure sustainable aquaculture. Usage of fishmeal in formulations in shrimp feed has been substantially reduced compared to the earlier days. Installation and operation of sophisticated fishmeal plants in India in recent years to produce quality fishmeal from the available fishery resources and the trimmings from the fish processing plants, which otherwise used as fertilizer in agriculture, is an emerging input resource for aquaculture feed industry. The installed capacity of fish meal production in the country is estimated to be 1,50,000 tonnes and out of which 100000 tonnes was actually produced during the year 2016. Fishmeal production in India is split into the production of sterile fishmeal of high quality (60-65% protein) from pelagic fishes such as sardines, wherein fish oil is also extracted as a high value by-product. In the other one where fishmeal prepared from processing waste (protein 50-60%), fish oil separation is not practiced. In addition, sun dried trash fish (176000 tonnes), mantis shrimp (57,940 tonnes), steam sterilised shrimp head and sergestid shrimp (48,000 tonnes) are also available for inclusion into aqua feeds. Squid waste meal is also available for use in aqua feeds. This prepared mainly from the waste (60000 tonnes) available after processing the squid for human consumption. The waste largely consists of head, fin, and viscera along with unclaimed mantles and tentacles. The protein content of the waste is high enough for proteolytic hydrolysis (enzymatic digestion) to generate large amounts of peptides and free amino acids responsible for feed attraction and growth stimulation. This waste is collected and steam sterilised and converted into squid meal. In a pilot scale project conducted at CIBA in collaboration with a firm M/S. Jass ventures Private limited, squid waste was converted into squid silage using organic acid and has been used as an attractant in organic shrimp feed for tiger shrimp with highly encouraging results.

Plant protein sources

Among the plant protein resources, soybean meal (SBM) is the major plant protein source used in shrimp feeds and about 10 MMT soybean seed has been produced in the year 2016 and the annual availability of soybean meal for feed use ranged from 6-8 MMT during the preceding six years. Soybean meal for feed use is available in three grades with highest grade viz., hi pro soybean meal containing a minimum of 49 % protein is mainly used in the shrimp feed formulation. The medium grade contains about 46-48% protein and the other grade contains about 44-46% protein. SBM is extensively used in the aqua feed for its better protein content and less variability in the nutrient concentration. The better solvent extraction process adopted during the meal manufacturing destroys majority of the anti-nutritional factors (ANF's) present in SBM and the heat process adopted during the pelleting and extrusion also inactivates the ANF's.

An array of other important indigenous plant protein sources viz., ground nut cake(1.3MMT), cottonseed meal(4MMT), sesame cake (0.1MMT), mustard/rape seed cake (3.4MMT), sunflower cake meal(1.8MMT), coconut cake, corn gluten meal and rice gluten provides an added inventory for the feed sector. The major constraints in using these plant protein sources are the presence of ANF's imbalanced amino acid profile and variability in the nutrient content. These plant protein are used mainly in the freshwater fish feeds and the use is very limited in shrimp feed formulations.

Energy sources

Cereals such as wheat, rice, maize, sorghum, other millets and cassava are cultivated extensively in the country. Wheat and its by-products and broken rice are the main energy sources used in shrimp feed while maize, broken rice, cassava and other millets are used in fish feeds. Rice bran and wheat bran are conventionally used in aqua feeds.

Non-conventional ingredients

Non-conventional aqua feed ingredients such as blood meal, meat meal, bone meal and poultry feather meal, poultry by-product meal, guar gum, gram chunies and by products from lentil processing industries and silkworm pupae are also available for use in aqua feeds. Vegetable oils such as groundnut oil, coconut oil, sunflower oil, sesame oil, mustard oil, soybean oil, soy lecithin, palm oil and, of late, rice bran oil are also produced in the country, which caters to the fat requirement.

Novel ingredients

Recently, Indian, aqua feed sector is exploring the opportunity to utilize abundantly available spent residues ,dried distillery grains with solubles (DDGS) as a potential ingredient in fish and shrimp feeds. DDGS available in two grades with 40-45% protein and 55-60% protein. In addition, de-fatted marine micro algal biomass generated from the potential biofuel production could be a potential ingredients due to their high levels of protein, relatively well-balanced amino acid profiles, and rich contents of minerals and vitamins, along with unique bioactive compounds. Indian aquafeed sector also started trialling some of the potential and novel ingredients such as insect meal, macro algal meal from freshwater, micro algal biomass, dry seaweed biomass, single cell protein etc.

ICAR-CIBA's initiative in cost effective feed for shrimp

ICAR- Central Institute of Brackishwater Aquaculture (CIBA) as nodal agency in research and development of coastal aquaculture is in the forefront in India in developing indigenous aqua feed technologies and products for different life stages of finfish and shellfish including shrimp. After extensive field evaluation these indigenous cost effective feed technologies are commercialized and made available for the farmers. Recent commercialization of indigenous shrimp feed for vannamei, which branded as Vannamei ^{Plus} (Figure 6) paved the way for availability of cost effective shrimp feed for small and marginal farmers, which provided a cost reduction of about 8 to 10%. As an approach to reduce the feed cost, we suggest establishment of small and medium feed manufacturing units, with a capacity of 1-2 tons/hr, which can cater to the requirements of certain clusters of shrimp/fish farming area, by tapping the locally available ingredients and resources. This model has become success and more such small and medium scale feed mills has to be established across the shrimp farming areas in the country. This would pave the way for affordable, quality feed for small and marginal farmers.

Farm made feed

Farm made feed so far are not a choice for vannamei by the farmers and not presently used for feeding the shrimp, vannamei. However this could be option in the traditional or extensive system of shrimp culture practiced in Kerala and west Bengal. This is not only cost effective, but also could judiciously use the locally available resources and generate employment and livelihood for small scale farmers.

Shrimp Larval feeds

Larval feeds for shrimp are not manufactured commercially in India and are imported and used. At present the larval feeds from multinationals such as Inve, Zeigler, SIS, Biomar, Tierarzt and Nutreco are used. CIBA has done extensive research in larval feed and has developed the larval feed for shrimp, CIBA Shrimp larvi ^{Plus} (Figure 7). This feed has been tested in the commercial vannamei hatcheries in collaboration with a private partner and the results showed on par performance with imported feeds. The main advantage of the Shrimp larvi ^{Plus} is the cost effectiveness and this feed is in the process of commercialisation.

Conclusion

Indian shrimp feed industry has rapidly evolved over the years and could cater to the need of fast growing shrimp aquaculture during the last five years. The state of the art facilities available in feed production, novel, scientific and innovative approaches in feed formulation, and improved methods of feed management would cater to the rapidly growing shrimp aquaculture sector. Substantial availability of feed ingredient in India would pave the way for sustainable shrimp feed Industry. More research have to be focussed on understanding the nutrient requirement at farm level, and the ~~and~~ industry- institute should work in tandem to meet the existing and emerging demands of the sector. Availability of cost effective and quality feeds to farmers and ways and means of reducing the cost of production of shrimp using efficient feed mill technologies, use of locally available fees ingredients, improved feed presentation and delivery and better feed management would assumes paramount importance in the years to come, to sustain the growth and sustainability of the vibrant Indian shrimp farming sector.

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Potential finfish species for brackishwater aquaculture – Opportunities, Challenges and way forward

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Finfish Culture Division

Introduction

Brackishwater aquaculture has been an age old practice in the traditional culture systems of India in the form of traditional prawn filtration systems *Pokkali fields* of Kerala, the *bheris* of West-Bengal, *Gheris* of Orissa, *Khar* lands of Karnataka and *Ghazni* fields of Goa. As early as 1911 James Hornell suggested the development of salt water fish farming in Madras Presidency which led to establishment of marine fish farm near Tuticorin by utilising few lagoons in the area and stocking mullets and sand whiting (*Mugil* spp. and *Sillago* sp.). The prime brackishwater finfish species having high consumer preference and market demand in India include Asian seabass *Lates calcarifer* (Bloch, 1790), grey mullet *Mugil cephalus* (Linnaeus, 1758), milkfish *Chanos chanos* (Forsskal, 1775), pearlspot *Etroplus suratensis* (Bloch 1790), and red snapper *Lutjanus argentimaculatus*. In the year 1997, a significant milestone achieved with respect to brackishwater finfish aquaculture in our country with the successful breeding of Asian seabass in captivity, at the Central Institute of Brackishwater Aquaculture. The R&D in seabass breeding thus led to the establishment of the first brackishwater/ marine finfish hatchery of our country located at CIBA, Chennai.

Subsequently seabass hatching and rearing technology, developed by CIBA was transferred to Rajiv Gandhi Centre for Aquaculture (RGCA) during 1999-2000 (CIBA Annual Report, 2000-01). The hatchery produced seeds are being produced and supplied to farmers, which is further spreading the seabass farming in country. Today private entrepreneurs are taking interest in the farming of seabass, and the demand for hatchery produced seeds of finfish species is on the rise, reflecting the increasing interest of brackishwater farmers in adopting seabass nursery rearing and farming technology. The year 2015 will go down in the history of Indian brackishwater aquaculture for another significant milestone achieved- the first successful captive breeding of the marine herbivorous fish species, the milkfish *Chanos chanos* by ICAR-CIBA. The herbivorous species forms the mainstay of brackishwater finfish aquaculture of many south-east Asian countries such as

Indonesia and Philippines. Captive breeding followed by successful larval rearing and fry/fingerling production helped to conduct scientific milkfish farming in India. Successful results came from the efforts for transporting the batches of fertilized eggs of milkfish to private entrepreneur and the subsequent rearing of milkfish larvae to fry stage and its sale to grow-out farmers. CIBA has been successful in breeding and successfully developing farming models of the catfish *Mystus gulio* which commands good market value in the states such as Bengal.

The declaration of pearlspot, "*Karimeen*" as the state fish of Kerala in 2010 came as a boon to this indigenous cichlid which has been an important brackishwater food fish of Kerala, with great local demand with a market price in the range of Rs. 300 to 600/kg. The policy helped to lay a new focus on the species leading to the revival the aquaculture for improving the overall pearlspot production in the state. Grey mullet *Mugil cephalus* by virtue of occupying lower trophic levels of the food chain and at the same time having a high market value is a species of significance for sustainable aquaculture systems like the IMTA (Integrated Multi-trophic Aquaculture) and finfish polyculture. We are still reliant on wild seed resources for its aquaculture the availability of which is today getting limited due to deteriorating nursing grounds and emerging regulations on wild seed collection by states like Kerala. Hence ICAR-CIBA has laid high impetus on developing a technology for grey mullet captive seed production. Brackishwater ornamental aquariculture is also given a special focus and being developed as new area on account of its potential to play a significant role in providing livelihood to small scale farmers and Self Help Group's, by adopting the homestead rearing model. CIBA has bred spotted scat, *Scatophagus argus*, moon fish *Monodactylus argenteus* for the first time in the country. Species such as orange chromide, crescent perch, pearlspot are also being developed as ornamental fish production models, suitable to get regular monthly incomes to farmers.

Brackishwater finfish species

1.1 Asian seabass, *Lates calcarifer*

Asian seabass is an important food fish in Indo-pacific region, most sought after candidate species for aquaculture in recent years and it has expanded as candidate species for cage culture and in the recirculating systems globally. Asian seabass *Lates calcarifer* is an euryhaline fish belongs to the family Centropomidae widely distributed in the Indo-West Pacific region, Arabian Gulf to China, Taiwan Province of China, Papua New

Guinea and northern Australia It is found throughout the northern part of Asia Southward, Queensland (Australia) West ward to East Africa ((Copland and Grey1987).

Taxonomy

Class:	Actinopterygii
Order:	Perciformes
Family:	Latidae
Genus:	*Lates*

Lates calcarifer is known as seabass in Asia and Barramundi in Australia and it has also been variously called as '*bhetki*' in India. For the first time in India, seabass was bred in captivity and successful larval rearing was done in 1997 which paved way for the large scale seed production and culture of seabass in India followed by year-round seed production by establishing recirculation aquaculture system. Hatchery production of seabass involves breeding of captive broodstock fish, rear fish larvae up to fingerling size with weaning feed. The hatchery technology includes, broodstock development, management, maturation, breeding, larval rearing, live feed culture and nursery rearing. Asian seabass is catadromous fish, grows in coastal low saline area migrate to sea for spawning, in the Sea many influential factor induces maturation and spawning process naturally. In captive broodstock, many times marine conditions may not prevail hence we have to induce maturation and spawning. The exogenous hormones are used to induce maturation and spawning, In CIBA, Leutinizing Hormone Releasing Hormone – analogue (LHRH-a) being used for maturation and spawning. For induction of maturation in the broodstock fishes reared for more than two years in the case of males and 3 years in females if found not matured, the hormonal pellet prepared with LHRH-a @50-100 µg/kg implanted to prolong the gamete formation.

1.2. Milkfish, *Chanos chanos*

Milkfish *Chanos chanos*) is one of the most popular cultivable brackishwater finfishes in the south East Asian countries and widely distributed in the Indo-Pacific region. The maximum weight and age of this fish were reported as 14kg and 15 years respectively. In India, it is named as *Paal Meen* in Tamil, *Pala Bontha* and *Tulli Chepa* in Telugu, *Poomeen* in Malayalam, *Hoomeenu* in Kannada, *Golsi* in Goa and *Seba khainga* in Oriya. Being herbivore, milkfish feeds on plankton, benthic algae, detritus matter in the natural condition and easily accepts the pellet feed under culture condition. It can tolerate and live in extreme salinity ranging from 0-100 ppt but growth is optimal between 0.5-40 ppt. Milkfish can attain the table size weight from 400 to 500 gm in 5-6 months under culture condition. Milkfish having tiny bones resemble with Hilsa and can be considered as a '*Decan Hilsa*'. Milkfish can be produced in the farm with the production cost of Rs. 80-90/kg by feeding with low protein pellet feed.

Kingdom:	Animalia
Phylum:	Chordata
Order:	Gonorynchiformes
Family:	Chanidae
Genus:	*Chanos*
Species:	*Chanos chanos*

ICAR-CIBA has made major breakthrough on captive breeding of milkfish for the first time in India during June 2015 and developed comprehensive technology package for seed production of milkfish. Captive breeding of milkfish involves development of land based captive broodstocks (6+ years old) and application of calculated dose of slow release hormone (LHRH-A) pellet. Hatchery produced seeds were distributed among farmers for promotion and demonstration of milkfish farming in coastal states. To educate the farmer community about this technology, routine trainings are being organized by ICAR-CIBA on need-based.

1.3. Grey mullet, *Mugil cephalus*

Mugil cephalus L. is cosmopolitan and contribute significantly to the economy of countries of Southeast Asia, Mediterranean region, Taiwan, Japan and Hawaii. This species is euryhaline and capable of surviving in wide variety of marine, estuarine and freshwater environments of varying turbidity, salinity and dissolved oxygen levels (Thomson 1955, Ibanez and Guitierrez-Benitez 2004).

Taxonomy

Kingdom	:	Animalia
Phylum	:	Chordata
Class	:	Teleostei
Order	:	Mugiliformes
Family	:	Mugilidae
Genus	:	Mugil
Species	:	*Mugil cephalus*
Common Name:		Striped mullet, grey mullet

The species is recognised economically as an important food fish. The roe of the species is used to prepare "*Bortaga cavier*" a delicacy in Taiwan and Japan and hence referred to as "Grey gold". In India grey mullet has good market in all the coastal states fetching between Rs. 300-400 per kg. Grey mullet is situated at the base of the food chain and feeds on detritus and benthic micro-algae thus playing its significant ecological role as a converter of primary productivity, particulate organic matter and detritus into quality fish protein. The significant market demand, tolerance to wide salinity ranges and ability to utilise the herbivorous and detrital food chain qualifies it as an excellent candidate species for aquaculture. A quality broodstock forms the foundation stone of a breeding programme. Being a high value herbivorous species, grey mullet has high potential to contribute to the brackishwater aquaculture production. Considering the economic significance of the grey mullet, the major constraints affecting its development has to be addressed scientifically, especially with respect to seed production. ICAR-CIBA has also initiated captive seed production of grey mullet seeds are being produced at the fish hatchery of CIBA experimentally for the past three years during the annual breeding period. Few interested farmers were supplied with seeds for farming. Males of grey mullets mature between 250-300 mm standard length while females mature at slightly larger size, 270- 350 mm. Males are reported to mature at approximately 2-3 years of size while females mature at 3-4 years. The stage of maturity in female fish is assessed by biosying the oocytes. This helps in judging the right stage of maturity and giving the appropriate hormonal treatment. Captive grey mullets found to possess an oocyte size about 80-90 µm were found to be in the primary oocyte stage, 110-120 µm in the slightly advanced stage of the primary oocyte, the perinuclear stage. Oocytes in the size range of 140- 150 µm were found to be in the cortical alveoli stage. Further, oocytes above 180 µm were seen to be vitellogenic. This stage is good for administering LHRHa implants for supporting oocyte development to functional maturity. In grey mullets, an ova diameter of 600 micrometre is reported to be optimum for successful induced spawning.

1.4. Mangrove red snapper, *Lutjanus argentimaculatus*

Mangrove Red Snapper *Lutjanus argentimaculatus* is an Indo-Pacific species that inhabits riverine, coastal and offshore reef habits. Juveniles and young adults found in mangrove estuaries and in the lower reaches of freshwater streams. They migrate offshore to deeper reef areas, sometimes penetrating to depths in excess of 100 m. The fish has greenish-brown to reddish body. Fishes that are found in deeper water have reddish body colour.

Young fishes have eight whitish bars on the sides and 1-2 blue lines across the cheek. *L. argentimaculatus* has a slightly concave caudal fin and the scale rows on the back are roughly parallel to the lateral line. Habitat frequently consists of areas of abundant shelter in the form of caves or overhanging ledges. It feeds mainly on fishes and crustaceans. *L. argentimaculatus* is an important food and sport fish throughout the Indo-Pacific region, but never found in large quantities. They caught mainly with hand lines, bottom longlines and trawls. Aquaculture importance of this species has been well documented due to high demand in the international market.

Taxonomy	
Phylum:	Chordata
Sub-phylum:	Vertebrata
Class:	Pisces
Sub-class:	Actinopterygii
Order:	Teleostei
Family:	*Lutjanidae*
Genus:	*Lutjanus* (Bloch 1790)

L. argentimaculatus can attain the maturity under pond/tank/cage based captive conditions when they maintained in the salinity regime of above 30 ppt. The fish can be induced to spawn through hormone (hCG) treatment. Lutjanus are broadcast spawner. Batch fecundity of this species estimated was around 5.0 lakh eggs/kg body weight. The size at first maturity for male and females reported were 2.5 and 3.9 kg respectively. The fertilized eggs size would be around 750µm and the newly hatched larvae size were between 1.6-1.75 mm. The larvae can reach to 2.0 cm fry size at 40 day post hatch by feeding with live feed such as rotifer Artemia nauplii followed by the artificial feed.

1.5. Pearlspot, *Etroplus suratensis*

Pearlspot, *Etroplus suratensis*, is a high value food fish popular in different coastal states of India; it is also finding market as an ornamental fish. Pearlspot is distributed in peninsular India and Sri Lanka. Its tolerance to wide range of salinities makes aquaculture of the species possible in both freshwaters and brackishwater bodies. Being omnivorous in nature, aquaculture of pearlspot is relatively simple, economical and especially suitable for small scale aquaculture for supporting livelihood of fish-farmers. Pearlspot is extensively farmed in brackishwaters of Kerala has shown productions upto 1t/ha when cultured with milkfish and mullets (George, 1971).

Taxonomy	
Class:	Actinopterygii
Order:	Perciformes
Family:	Cichlidae
Genus:	*Etroplus*
Species:	*suratensis*

Traditionally pearlspot has been cultured in pokkali fields of Kerala along with other brackishwater fishes. Pearlspot has chiefly been cultured by farmers as a component of polyculture in brackishwater systems. Small scale cage based aquaculture experiments showed that stocking pearlspot @ 200 nos m^3 in 2 m net cages can give a production of 26 kg m^3 in 200-260 days using commercial feed (crude protein-20%). More recently with the support of the state fisheries department many farmers and Self-Help Groups (SHG's) in Kerala are involved in culture of pearlspot in small cage (2-3 m^3) and pond systems. However, one of the major limiting factors for expansion of pearlspot aquaculture is inadequate availability of seed for stocking in different culture systems. Pearlspot exhibits a high degree of parental care and has very low fecundity as compared to other brackishwater fishes. These are the main reasons which makes mass scale seed production of the fish challenging. Hence development of technologies which allow seed production at multiple locations in the form of backyard hatcheries or small scale seed production systems is important. However, the fish is easier to breed compared to many other brackishwater fish and today different models in a range of systems are available or being tested, so that seed production can be conducted by entrepreneurs, Self- Help Groups or farmers themselves depending on their local resources.

1.6. Long whisker catfish, *Mystus gulio*

The long whiskers catfish, *Mystus gulio* (Hamilton, 1822), belongs to the family Bagridae is a euryhaline fish, which is commonly called as '*nona tengra'* in Bengali (Kumar et al., 2019). *M. gulio* is commercially important estuarine catfish of Sunderban delta of Bangladesh and India. It has also market value in state of Andhra Pradesh and Odisha. It is a small indigenous fish species (SIS), having high nutritional value (Ross et al., 2003). The domestic market price of *M. gulio* is very high and ranges from 200-700 Kg-1. The important attributes such as high nutritional value, consumer demand, high market price, hardy nature and faster growth make this species a desirable candidate species for aquaculture in Southeast Asia. Due to euryhaline nature this fish can be breed and farmed in both fresh and brackishwater environments (Siddiky et al., 2015). This species is suitable for co-cultured with other brackishwater fishes in paddy fields and bheris of the Sunderban. This fish can also be farmed at high densities in cage and Recirculatory Aquaculture System (RAS). Expansion of *M. gulio* is stumbled because of the unavailability large quantity of hatchery produced seeds. In this connection, Kakdwip Research Centre of ICAR-Central Institute of Brackishwater Aquaculture, West Bengal, India has developed and popularized a cost effective, farmers-friendly seed production and farming technology of *M. gulio*.

Taxonomy	
Class:	Actinopterygii
Order:	Siluriformes
Family:	Bagridae
Genus:	*Mystus*
Species:	*M. gulio*

During the spawning season, mature *M. gulio* are collected from the broodstock ponds. An ovarian biopsy of the female is performed to assess maturity. However, without ovarian biopsy, maturity can be judged through morphological observation of vent; a swollen belly and swollen reddish vent indicates maturity. Mature males can be identified by the presence of elongated papillae with a pinkish tip. Generally, females and males in the size range of 60-120 g and 25-75 g, respectively, are selected for breeding. The operational sex ratio of males and females is 2:1. A single intramuscular injection of either human chorionic gonadotropin (HCG), leutinizing releasing hormone (LHRHa) can be used to induce the fish to spawn.

Brackishwater Finfish aquaculture- The Present Scenario

Asian seabass has been mooted as a prime candidate for diversification to finfishes on account of its high market demand (Rs. 400-600 per kg), and availability of hatchery produced seed and formulated extruded feeds. Globally seabass is cultured in different culture systems like cage, ponds, pens and tanks showing the adaptability of the fish to the different culture methods. In India seabass is primarily cultured in brackishwater pond systems where it exhibits growth rates ranging from 800g-1.0 kg in a period of 6-8 months Partial harvesting of larger fish is carried out periodically in this extensive farming practice. Monoculture of seabass is practiced at stocking densities of 4000 to 5000 nos/ha^{-1} (initial wt.- 60-80 g) and a production of 3.5 to 4.0 t ha^{-1} can be achieved. The cost of production is around Rs. 175-225/kg at a fish sale price of Rs. 350-400. To develop a holistic model of seabass culture, a formulated seabass feed, 'Seabass^{Plus}' has been developed by CIBA for nursery and grow-out culture which gives an FCR of around 1.5. Presently, seabass is considered as one of the most potential finfish aquaculture candidates for those looking for diversification from shrimp culture.

Herbivorous species form the backbone of sustainable fish farming models due to the lower cost of production- milkfish, grey mullets and pearlspot have been traditionally cultured by enhancing the ponds natural productivity and through low cost supplementary feeding using agro-by-products. CIBA's effort on the seed production of these fish species bore fruit with the captive breeding of milkfish at its Muttukadu Experimental Station (MES). Today hatchery produced milkfish seeds distributed to brackishwater farmers across the country are being cultured using grow-out formulated feeds specially developed for milkfish. Interventions in parental care of pearlspot have assured enhanced seed production and given rise to a modular tank based system for seed production for adoption by small scale farmers.

Novel finfish farming technologies being given key focus by CIBA

In keeping with its slogan "Brackishwater aquaculture for food, employment and prosperity", ICAR-CIBA recognizes that brackishwater aquaculture as a powerful tool to bolster livelihood and nutritional security. Hence, focus has been placed by CIBA to develop location specific need based models for different stakeholders.

i) **Satellite based nursery rearing of seabass** - One of the interesting innovative approaches adopted by CIBA for developing seabass as a separate livelihood activity is the hapa based nursery rearing model where hatchery reared fry are cultured for a duration of about 60-75 days to fingerlings size. This model is being mooted both as a livelihood activity for farmers for giving returns in a short duration and also for developing satellite seabass seed rearing centres to facilitate widespread adoption of seabass aquaculture. This helps in saving space and time during the grow-out culture of seabass and helps farmers to tide over the phase which requires physical labour for frequent size grading of the fry. Adoption of nursery rearing of seabass fry by farmers involved in low volume cage culture of Asian seabass *Lates calcarifer* is encouraged by ICAR-CIBA. The activity helps farmers get a better control over the initial size used for stocking in cages, a factor critical for getting optimum survival rates and fish production.

ii) **Low volume cage culture** - The access of small scale farmers to diverse opportunities offered by different aquaculture initiatives is often limited by ownership or access to water resources, access to simple and adoptable technology and high investment costs. Production of high value fish using low volume cages set in brackishwater bodies can thus be a potential livelihood option to the poor. Low volume cages can be fabricated by the farmers themselves. Species like seabass stocked at rates of 25 advanced fingerlings/r cubic-m has shown to yield a production upto 20 kg m^{-3}. For example, in a demonstration using 3 cage units of 8 m^3 each, a production between 450-500 kg of seabass was obtained partial harvest of the fish from the cages can thus provide the family with sustained monthly income of Rs 10,000-15,000 by sale of the fish at Rs 400/kg. Construction and setting up of these low volume cages is also being mooted as a skill development activity for small farmers.

iii) **Integrated Multi-Trophic Aquaculture (IMTA)** - IMTA is the farming of aquaculture species from different trophic levels and with complimentary ecosystem function. For laying a roadmap for sustainable aquaculture, CIBA is developing economical polyculture models and adopting IMTA (Integrated Multi-Trophic Aquaculture) approach. These models have been successfully demonstrated at Kakdwip, West Bengal and Sindhudurg, Maharashtra with farmer's participation. The benefit cost ratio of pond based IMTA was worked out to be 1.5 as compared to 1.4 in monoculture of shrimps at the culture demonstrated at Sindhudurg district of Maharastra. Culture demonstrations at Kakwdip centre of ICAR-CIBA have shown the environmental and economical benefits of IMTA over conventional culture practices.

iv) **Finfish seed production from egg stage** – Realising the need for elaborate investment and infrastructure for maintenance of broodstocks of finfishes, private entrepreneurs are encouraged to transport fertilized eggs for hatching and subsequent larval rearing in their hatcheries. This model has been successfully adopted in case of candidate species like seabass and milkfish by private entrepreneurs.

v) **Ornamental fish seed rearing as a household activity** - CIBA is mooting the adoption of nursery rearing of ornamental fish like silver moony, spotted scat orange chromide and pearlspot as an activity to be adopted at household levels for getting regular monthly income. As a part of the *Mera gaon mera Gaurav* program of CIBA, pearlspot nursery rearing as a livelihood activity is being successfully adopted by tribal women groups.

Way forward for brackishwater finfish aquaculture development and CIBA's interventions

CIBA has placed a major thrust on developing economically viable seed production technologies of the prime brackishwater candidate species. The development of hatchery technologies are also being followed by partnership with entrepreneurs for facilitating technology adoption in different states in PPP mode, this is witnessed in the partnerships both for shrimp and finfish species. There is a need of private sector hatcheries for scaling up of seed production for catering to the increasing demand of fish seed among brackishwater fish farmers. For the development of large scale economical finfish aquaculture practices, development of efficient, eco-friendly and low cost feeds is perceived as the next major challenge. CIBA has developed feeds for Asian seabass and other major species are being tested. By entering into partnerships CIBA is also facilitating the development of feed mills and developing feeds for different brackishwater food fish and ornamental species. Considering the significance of aquaculture as a tool to alleviate poverty, provide livelihood and nutritional security, CIBA lays a major thrust on developing family farming models for widespread adoption in different states. Thrust is also being laid for developing suitable marketing models for getting the best price for the farmer.

The aquaculture industry in the inland saline waters needs an ecological mapping of salt affected areas (including salinity, composition) for development of region-specific aquaculture practices. Introduction of low-cost, low-risk species for sustainable development of inland saline water aquaculture, with special reference to small and poorer farmers is need of the hour. Establishment of a National Aquaculture Network and Public Private Partnerships (PPP) to ensure the supply of inputs like seed, feed and others, and support for marketing, processing and exports to non-coastal states. Development of production skills and farming clusters, promoting support groups, cooperatives and contractual farming under strict biosecurity monitoring and regulatory governance for the development of brackishwater finfish aquaculture in the inland saline areas. CIBA visualizes and strives for a holistic sustainable development of the brackishwater aquaculture sector of India with an underlying thrust on sustainability, economic viability and livelihood provision. For this CIBA reaches out for active partnerships with the state government, the private sector, other research organisations and Self-Help-Groups to develop and advance the brackishwater aquaculture technologies for the betterment of the farming sector. Species diversification in aquaculture and developing need based location specific technologies is the best roadmap for sustainable aquaculture sector for our country.

Indian shrimp farming sector and international and domestic demand for shrimp

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Brackishwater aquaculture in India was in vogue as traditional practice in *Pokkali* paddy fields along the Kerala, traditional earthen ponds of Orissa and Goa, *Bheries* of West Bengal. Traditional aquaculture systems facilitated mostly one crop only in a year. The feed used was natural feed like rice bran and oil cakes, and the crop season was usually from December to April. No diseases were reported particularly. The yields were low, averaging around 150 to 200 kg/ha. The shrimp exports consisted mostly of sea capture and culture contributed negligible quantities.

Brief timeline of development of Indian shrimp farming

The 1970s started with a new hope and fervor. Scientific shrimp farming started in the country with the work of Experimental Brackish water Fish Farm in Kakdwip, West Bengal, by the Central Inland Fisheries Research Institute under the Indian Council of Agricultural Research (ICAR) in 1973. Concurrently, shrimp seed production studies were initiated in Narakkal, near Kochi, in Kerala, by the Central Marine Fisheries Research Institute of ICAR. Narakkal village, under the Cochin-based Central Marine Fisheries Research Institute, which did pioneering investigations into shrimp breeding and seed production. Indian Council of Agricultural Research started an All-India Coordinated Research Project on Brackish water Fish Farming (AICRP on BWA) in 1975, with centres in West Bengal, Odisha, Andhra Pradesh, Tamil Nadu, Kerala, and Goa. The feed technology was improvised with preparation of farm made feeds as balls or pellets using fish oil, fish by catch and other plant sources like Ground nut oil cake etc. The diseases reported were bacterial and fungal which were effectively cured by application of recommended chemicals. The yield were 1000-1500 kg/ha.

In the late 1980s, progressive farmers started import of technology from South East Asian Countries along with technicians. The use of machineries like aerators came into existence. Imported pellet feed were found to give higher growth and better yields. Semi-Intensive farming was initiated in many pockets of Andhra Pradesh and Tamil Nadu. The AICRP on BWA became an entity itself as ICAR - Central Institute of Brackishwater

Aquaculture on 1st April 1987. In the same year, Hindustan Lever's Sandeshkali unit in West Bengal achieved the 3.5 t/ha/crop and Victory Aquafarm, Tuticorin harvested a yield of 8 tonnes per ha of Indian white shrimp, with use of imported pellet feed. These reports ignited the interest of aqua-farmers in shrimp farming. In the late 1980s, MPEDA established the Andhra Pradesh Shrimp Seed Production Supply and Research Centre (TASPARC) based in Andhra Pradesh and Orissa Shrimp Seed Production Supply and Research Centre (OSPARC) based in Orissa which provided assistance and paved the way for the establishment of a number of private hatcheries. The farmers used imported pellet feeds and were happy with the performance. Though bacterial, fungal, and parasite issues were there, the situation was manageable. India started getting attention from major import destinations of the USA, Europe and Japan.

The decade of the 1990s started with the grand entry of tiger shrimp (*Penaeus monodon*) in the aquaculture space of the country. A semi-intensive culture technology was demonstrated in a pilot-scale project by the MPEDA funded by the Department of Biotechnology. The semi-intensive farming technology demonstrated production levels reaching 4–6 tonnes/ha. Credit facilities from commercial banks and subsidies from the Marine Products Export Development Authority helped to boost the shrimp farming sector. The corporate sector jumped in and NABARD, Insurance companies supported the industry assiduously. In addition, a number of Central Sector development schemes were initiated; including setting up Brackish water Fish Farmers Development Agencies (BFDA) in the maritime states for the development of shrimp farming. This paved the way for the establishment of a number of shrimp hatcheries and farms in the coastal states in the early nineties. Foreign feed and chemical inputs were relied upon mostly. India witnessed a extraordinary increase in the area under shrimp farming which occurred till 1995. The legal imbroglio and dreaded White Spot Syndrome Virus (WSSV) brought stellar growth to a grinding halt. Many of the major shrimp producing countries of Thailand and other South-East Asian nations, multiple shrimp diseases were reported. The global production started dwindling. All efforts were put to contain shrimp diseases. India could ensure the survival of shrimp farming against many odds, with the use of Polymerase Chain Reaction (PCR) screening of seeds and cluster farming with the adoption of Better Management Practices (BMPs)

The period 2000 to 2010 saw the continuation of revival efforts which partly helped, Farmed shrimp production recorded over a five-fold increase from 28,000 tonnes in 1988-89 to 1.5 lakh tonnes in 2006-07. The lull in the first decade of the new millennium was broken by the introduction of Specific Pathogen Free - Pacific white shrimp; *Penaeus vannamei* (SPF- PV) in 2009, after intense debate and conduct of a scientific import risk assessment study lead by ICAR-CIBA. The brooders were quarantined and vertical transmission of OIE listed diseases was prevented. The crops were successful with farmers' adherence to bio security measures. Feed sector saw the adoption of indigenous feed technology from ICAR-CIBA to an extent of 5-10% of the total area. Indian feed companies started performing well. The growth rates were high. The stocking densities were restricted and many farmers took a harvest of 8-10 tonnes and average national productivity rose to 4 – 5 tonnes. The introduction of SPF-PV helped the aquaculture sector to increase the production by 4.6 times from 1.5 lakh tonnes to 7 lakh tonnes compared to the pre-SPF-PV scenario.

Review of current state of shrimpfarming in India - the last decade

India's shrimp farming growth is remarkable since introduction of SPF vannamei (Fig. 1). Higher stocking densities, lower incidence of diseases and better animal growth of SPF *P. vannamei* made the farmers to adapt it faster and today 90 percent of Indian shrimp production is *P. vannamei*.

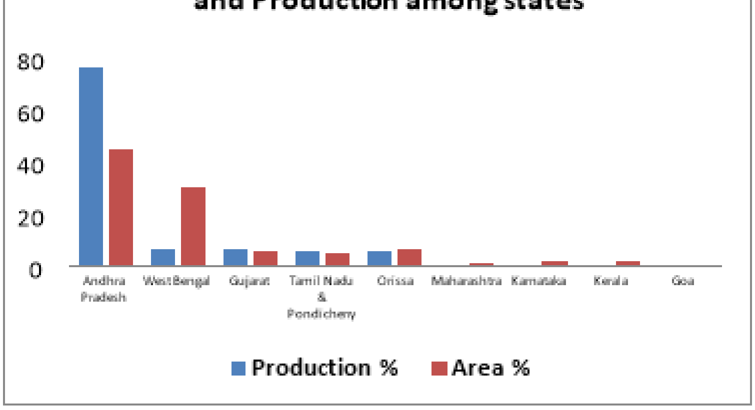


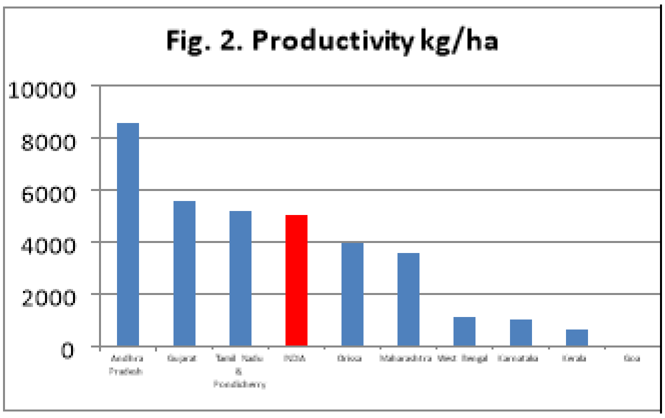
Fig. 1. Indian shrimp production 2021- Share of Area and Production among states

Base data is from MPEDA 2022 (www.mpeda.gov.in)

India's shrimp farming area was reported as 1,66,722 hectares with a farmed shrimp production of 8,43,633 MT in 2020-21. Andhra Pradesh has been the leader of aquaculture in India (Fig.2). The stste has a right mix of land water and capital resources to take up shrimp aquaculture production in the country.Society of Aquaculture Professionals (SAP) identfy the reasons of spectacular growth in shrimp farming in the state as

"…. While black tiger shrimp have been produced in low-salinity waters in some of the districts of Andhra Pradesh, vast expansion of shrimp production occurred in these districts in the last decade by way of constructing new ponds or using ponds previously used for fish farming".

Shrimp farming also expanded in two other states, notably in the states of Gujarat and Tamil Nadu as could be seen from Fig.2. These three states surpass average Indian shrimp productivity per ha.



Base data is from MPEDA 2022 (www.mpeda.gov.in)

Backward linkages

Industry estimates a surplus feed mill capacity in the country. There are 38 feed mills in India that can manufacture shrimp feeds, with an established production capacity of 3.5 million MT. In 2019, the volume of shrimp feed sales was estimated at 1.3 million MT. Indian shrimp farming industry reportedly, has an established production capacity of 120 billion postlarvae (PLs) per year, from an estimated 550 to 600 hatcheries. India produced 7 billion PLs in 2019.

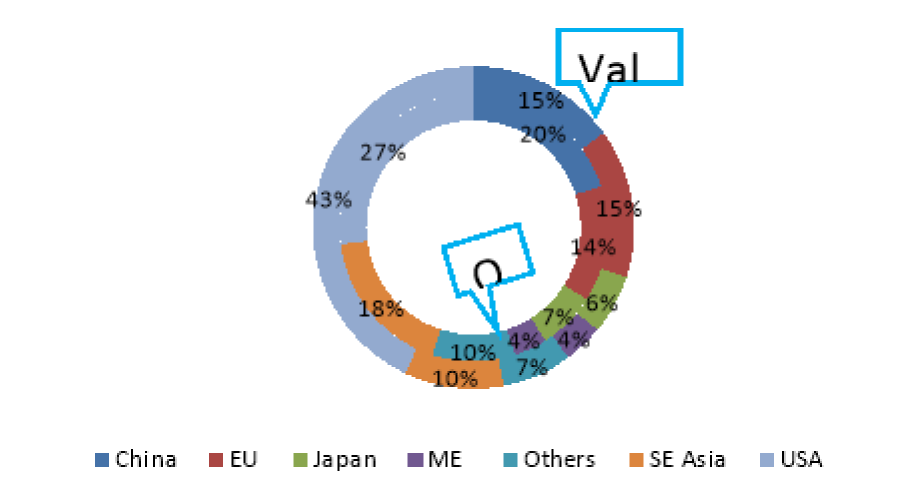
Production issues

Diseases continue to be the primary challenge to the productivity and profitability of shrimp farming in India. While WSSV is frequently detected, many farmers feel that it can be managed, and they do not feel as threatened by it as in the past. On the contrary, the occurrence of new diseases such as White Feces Disease and Running Mortality Syndrome are dreaded more because their causative agents have not yet been identified. The presence of the microsporidian parasite Enterocytozoon hepatopenaei (EHP), which results in the slow growth and size variation of shrimp, is also not definitively established.One of the responses of the shrimp farming community to these new diseases is to deploy nurseries where shrimp postlarvae (PLs) can be stocked at high densities and reared to about 0.5 to 1 gram each in size and then transferred to grow-out ponds. However, this method also carries the risk of pathogens infecting the shrimp in the nurseries and spreading to the farms. Thus, biosecure facilities and practices are required for the nurseries to fulfill their purpose of deployment.Shrimp nurseries are used as an intermediate grow-out stage between the hatchery and the farms, rearing the PLs to 0.5 to 1 gram each.

Consumption bottlenecks

On the demand side, marketplace diversification is high on the list of requirements. India’s shrimp exports are dependent on the United States and China in 2021 (Fig. 3). To reduce the overdependence on these two markets, India needs to increase its market share in other markets, particularly the European Union and Japan, each of which accounted for nearly one-third of India’s exports in the *P. monodon* days (SAP, 2021).

Fig. 3. Market wise export % of Quantity and Value



Base data is from MPEDA 2022 (www.mpeda.gov.in)

Through extensive campaigning and stringent monitoring, the presence of antibiotic residues has been reduced significantly in India’s shrimp supply, boosting its appeal to global markets.

Domestic market to be strengthened

Resource poor farmers resort to partial harvest after 60-70 days to purchase feed and other inputs for continuing their culture. Domestic markets also come handy to the farmers resorting to emergency harvest of undersized shrimps after noticing diseases or forecast of natural calamities. There is no organized data collection about farmed shrimp being sold at domestic markets. India's domestic market for shrimp consumption is estimated as less than 50,000 MT to 70,000 MT per year. Development of domestic market could lead to a significant increase in shrimp consumption. The rise of middle class with purchasing power, the large population of young people, and the health benefits of seafood could be the drivers of deveopment of a large domestic market for shrimp in India.

Future prospects for Indian shrimp farmers

India remains a competitive supplier of value-added shrimp to the world due to its low cost of labour and the scale of economy. India has managed to achieve by becoming one of the largest global producers of farmed shrimp. The established capacity of hatcheries, feed mills and processing plants will support future expansion.Compared to the major suppliers of shrimp in Asia, India has largely remained as a low-density producer, with a standard of about 40 shrimp per square meter widely adhered to.

In the northern states like Gujarat , where climatic factors restrict shrimp culture to a mostly one crop a year, farming of black tiger shrimp is seen as a viable option.The production of large *P. monodon* is considered a significant opportunity, (SAP, 2022). The government has recently approved the import of SPF black tiger shrimp into India (CAA, 2022) and post-larval SPF black tiger shrimp are already available in the market. However it is to wait and watch about the price advantage in international market for long duration tiger shrimp production.

India's future in shrimp production will depend on its ability to maintain its competitiveness in value addition, market expansion and application of production technologies that can keep diseases under control and help strengthen the industry.

References for updates:

1. CAA, 2022. https://caa.gov.in/hatcheries_approved.html
2. MPEDA, 2022. https://mpeda.gov.in/#
3. SAP, 2022. https://www.aquaprofessional.org/
4. SEAI, 2022. https://seai.in/
5. ICAR-CIBA, 2022. Aquastat http://www.ciba.res.in/aquastat/
6. FAO of UN, State of World Fisheries and Aquaculture, 2022. https://www.fao.org/publications/sofia/2022/en/

ICAR-CIBA mobile applications for efficient shrimp farming

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Introduction

Shrimp farming is technology intensive and high investment food production system. Shrimp is a high value commodity alone contributed 74% of Indian seafood export earnings worth Rs. 32, 521 crores in 2020-21. Introduction of Specific Pathogen Free (SPF) Pacific White Shrimp (*Penaeus vannamei*) in 2010 has multiplied the Indian shrimp production from 0.15 MMT in 2008-09 to 0.853 MMT in 2020-21 with an enhanced average productivity of 4.0 tonnes/ha (MPEDA, 2021). The profitability of shrimp farming is threatened due to high input costs, susceptibility to diseases, reduced farm gate prices and other production risks in the form of pond driven stress factors. Therefore, farm advisory services are important to enhance the technical capacities of the farmers to adopt appropriate farming practices and facilitate them to access quality inputs, diagnostic services and premium market. It is estimated that return to investment on farm advisory services is at 58% and attributed for increased production and household income of farmers to the tune of 18-30%. In India fisheries is the provincial subject, hence, the states have the major responsibility in providing extension advisory services. Though fisheries and aquaculture contribute significantly for food production, employment generation, societal development and national economy, it has not been adequately supported with a formal dedicated extension service at states level.

The Departments of Fisheries (DoFs) of states due to their limited reach, welfare-centric functions, lack of manpower, extension service orientation and budgetary constraints, could not perform this role efficiently. Many ICT aided projects were undertaken to provide the extension support, but due to their narrow focus and limited geographical attention they could not make an impact. Nevertheless, development of mobile networks that support greater data speeds and connectivity even in remote geographies and affordable prices of mobile handsets across the globe facilitated the exponential rise of mobile applications to bridge this communication gap in a required mode to the end users and facilitate research, extension, farmers, input and market integration. Studies have asserted that mobile phone based information pathway could ameliorate the major impediment, the access to farm advisory, for raising agricultural productivity among smallholders. Further, mobile applications were found to have ensured bidirectional information flow, customised advisories to the farmers, broken information asymmetry and enhanced knowledge level among the farmer segments. Application of mobile learning (e.g., apps) was more effective on end-user's knowledge than the use of traditional teaching approaches, due to the availability of the device without the restrictions of time and place. Shrimp farmers are constant information seekers from online sources and positively receptive towards accessing technology information through mobile applications.

ICAR-CIBA mobile applications for shrimp farming

In the present case, smart shrimp farming refers to facilitating shrimp farmers with specific mobile based applications inclusive of digital technology advisories, input optimizing calculations, bio-mass/stock assessment, on-farm disease diagnosis, farm risk assessment, pond-wise digital record keeping of all the farming operations, graphical display of pond parameters in the dash board, recommendations based on the data given by the farmer/end user for the efficient management of farms. In this context ICAR-CIBA has developed two android mobile applications viz., *CIBA ShrimpApp* and *CIBA ShrimpKrishi* both are available in the Google play store for free of coast. The applications developed adopting the Software Development Life Cycle (SLDC) approach which is comprised of eight phases and relevant methodologies as given in Table-1.

CIBA ShrimpApp: This app has eight information modules *viz.*, better management practices of shrimp farming, quantification of inputs, on-farm disease diagnosis, on-farm risk assessment, Frequently Asked Questions (FAQs) in shrimp farming, regulations, advisories and updates and posting queries were integrated in the mobile application. The internal consistency and validity of the modules were evaluated with appropriate reliability tests and judgement validation by domain subject matter specialists. The contents of the modules were translated in to programming language wherein the programme specifications were converted in to software instructions. Android Studio version 3.4.2 Integrated Development Environment was adopted for the development of mobile application with Java language as front end and the data bases were created as back end through Structured Query Language (MySQL). The framework for knowledge representation the mobile application along with the modules is given in Fig. 1.

Table 1: Software Development Life Cycle (SLDC) approach and methodology

Sl. No.	SDLC Phase	Subject matter	Methodology
1.	System Analysis	Shrimp aquaculture sector, production systems, exiting information flow, mobile application for bidirectional flow of information, shrimp farmers profile analysis, information need assessment, formats, receptivity and sustainability.	Farm survey, focus group discussions.
2.	Feasibility Analysis	Availability of technical content, subject matter specialists, operational resources, time and budget requirement.	subject matter specialists
3.	Requirement analysis	Availability of mobile networks, connectivity, access to smart phone by end users, technical information requirements, modules, format of delivery, preferred platform and language.	Farm surveys using structured questionnaire and focus group discussions.
4.	System design	Modules and content: static/dynamic; end user access to app, login details, dashboard details, navigation details, module choosing, accessing the content and interacting with modules.	Flow chart analysis
5.	Coding	Translation of module content in to programming language and software instructions. Operating system - Android Studio Integrated Development Environment with Java language as back end score and the data bases were created through MySQL.	Data base creation and linking, Android application file formatting and computer programmes.
6.	Testing	Testing to recognize the gaps, errors and missing necessities *vis-à-vis* the actual requirements. Unit/module wise testing for its functionality, integration testing	Content validation by domain subject matter specialists. Internal consistencies and validity

		for connectivity of modules, programme testing for coding and the whole app testing to ensure the user requirements. Each module interface of the app was tested to ensure its proper functioning.	of the modules evaluation with appropriate reliability tests.
7.	Implementation	Naming the mobile app “CIBA ShrimpApp” and display in the Google play store publisher for publication. Tutorial for end users.	Awareness / sensitization workshops; social media and online communication.
8.	Maintenance	Review of module contents for updation and modification, design and visual improvements.	Content review and updation analysis.

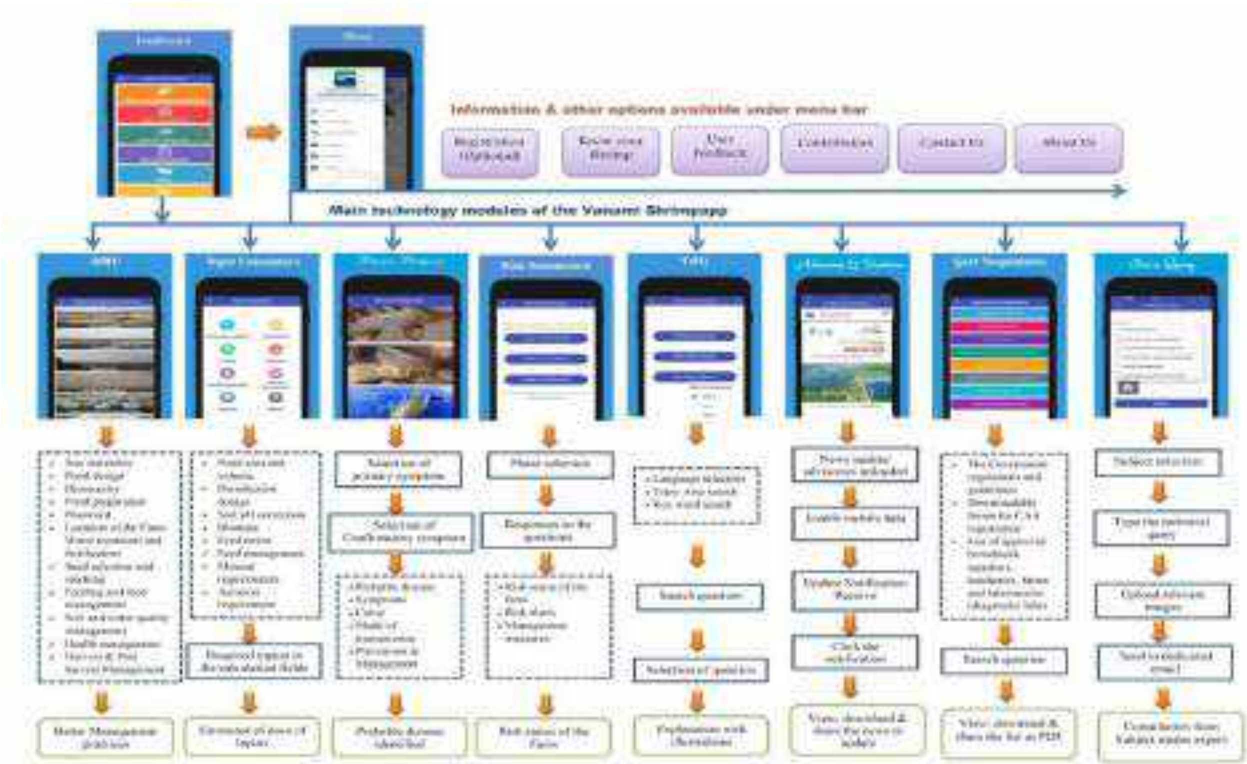


Fig-1. Framework for knowledge representation of CIBA ShrimpApp

CIBA Shrimp KrishiApp: The farmer end-users preferred that a mobile application in which the farmer or the farm operator could enter his/her data on water quality parameters, feed requirements, feed rationing, feeding behavior and feed management, biomass estimation, animal behavior, pond conditions, average growth, body weight and economics of inputs. The application could include blocks of farmer specific details, farm specific information, crop specific information and day-to-day data entry register. The application should integrate the data from these components, process them and show inferences in the form of graphs or data matrices using that the farmer can visualize the status of his farming and might take decisions appropriately. It is intended that by using this application the farmer can forecast his inputs requirements and productivity. Such that we can pool the data from several farms and forecast the requirements and identify the shortfalls in every aspect monitored and alert the farmer to rectify the issue and take an appropriate decision. Therefore the second application CIBA Shrimp Krishi was developed. A frame work for the mobile application is developed for implementing a mobile application for shrimp farm management (Fig-2). The screen shots of the mobile application are presented as figures below.

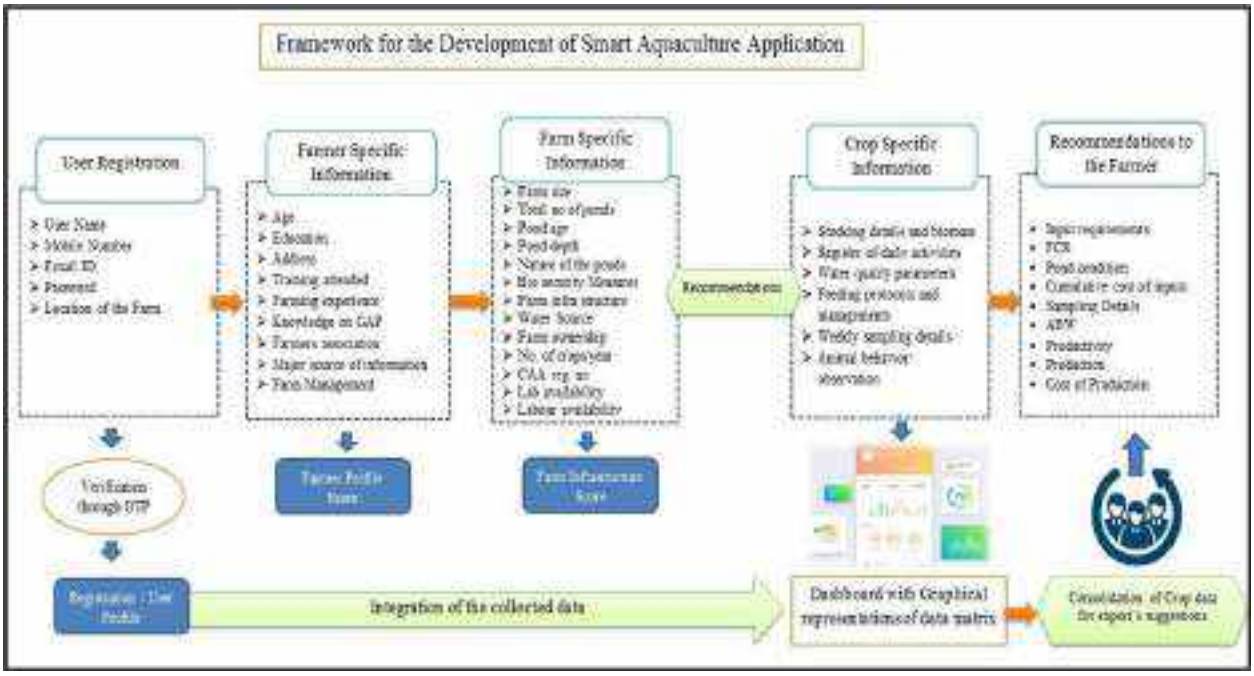


Fig-2. Framework for Mobile application for shrimp farm management

"CIBA Shrimp KrishiApp" was developed for handholding the farmers to make real-time based informed decisions at the farmer level. Front end was developed on Android platform SDK using Java programming language. This technology was used because it is portable to all android devices. Linux, Apache, MySQL and PHP tools were used for designing back-end. These tools are selected as they are open source, robust, and institute has the entire necessary infrastructure to store, manage and update the back-end content. The app size is 8 MB and it will work in Android version 5.0 and above. The app is made available in English, Hindi, Tamil and Telugu languages. Using this interactive mobile application, the farmer can input his farm data on day-to-day farming operations/observations from stocking to harvest. Based on the inputs provided and inbuilt decision-making system, the app will display pond-wise status on shrimp survival, biomass, feed conversion ratio, pond water quality, and the expenditure incurred (Fig-3 and 4).

Fig-3. CIBA ShrimpKrishi Mobile Application

Dash board: The dash board contains four dynamic modules they are: One time entry, daily entry, recommendations and post a query. Based on the number of ponds given in the baseline data, the pond numbers will be shown in the dash board and if the end user wants to add the pond number, then, he has to go back to the basic farm details and modify the same. Besides these modules four graphs are also inbuilt in the dash board. However, the graphs can be seen only after 15-20 days of entering pond wise details in the above modules.

Three expert systems viz., Shrimp feed management, water quality management and shrimp disease management are inbuilt in to the app. Based on the data fed in to the app, it Shrimp Krishi alerts the end-user farmer with technical advisories whenever any deviations are noted in the pond operational and critical day-to-day parameters such as water quality, feeding and shrimp health. The app can store the entire crop data in it, and the farmer can retrieve the data for their own long-term decision-making purposes or share it with their resource person for technical advice. Moreover, it paves the way for accessing real-time bulk data from the remotely located shrimp farms to monitor and extend customized technical advisories.

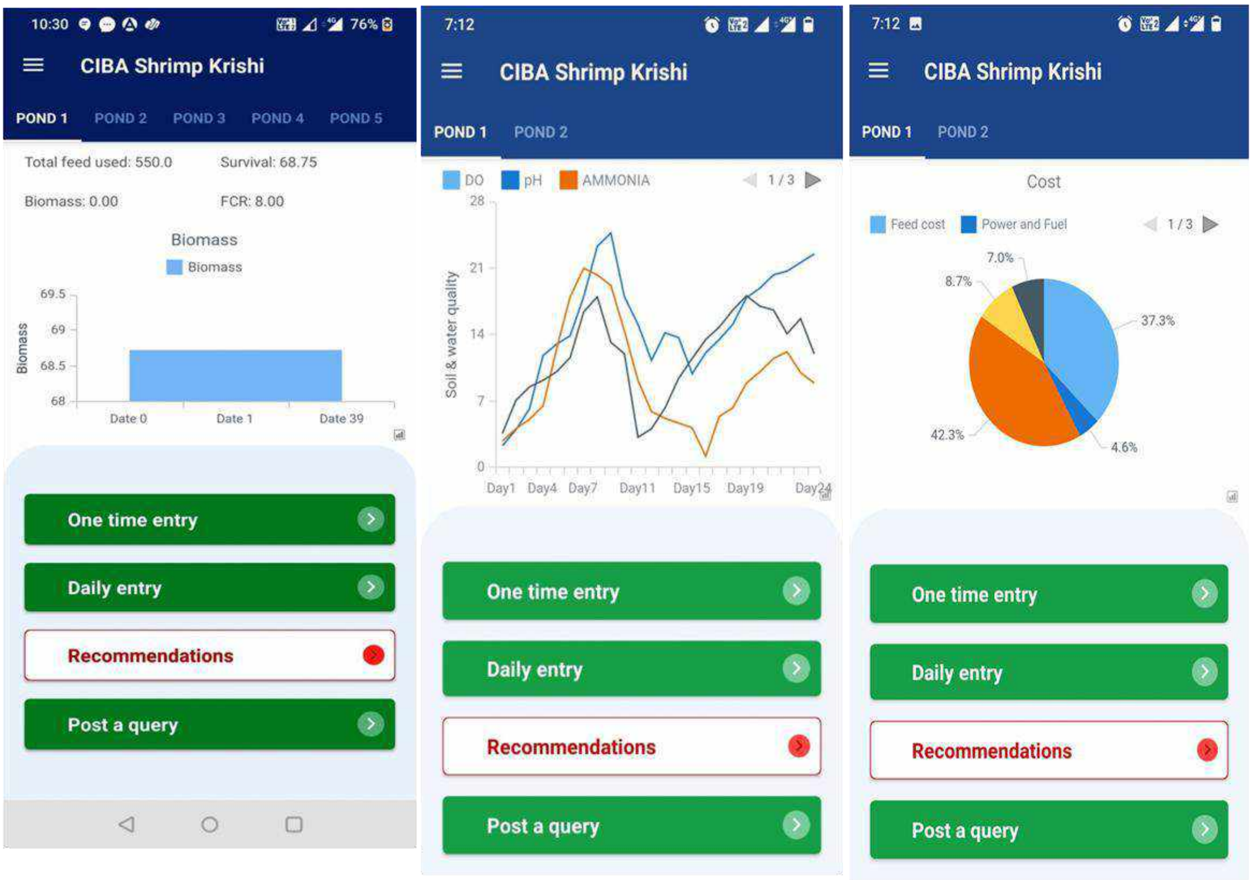


Fig-4. Graphical recommendations of shrimp pond parameters in the App

Impact of the mobile applications

The CIBA ShrimpApp has more than 29,000 cumulative downloads across the world (Fig-5) and rated as 4.5 out of 5.0. The application was found to have improved the knowledge level of end users to the tune of 20-37%. The Google firebase application data showed that 98.4% of users of CIBA ShrimpApp were free from errors and crashes. Through post your Query option more than 5,000 queries received and advisories were given the end-users (Fig-6 and 7). An evaluation study conducted among sample regular users indicated that the app aided in farm decision making and its design functionality and extension service function were perceived to be efficient. Considering pervading mobile connectivity and affordability of mobile phones, smart phone based mobile applications and data analytics would play a significant role in shrimp farm advisory services and its sustainability.

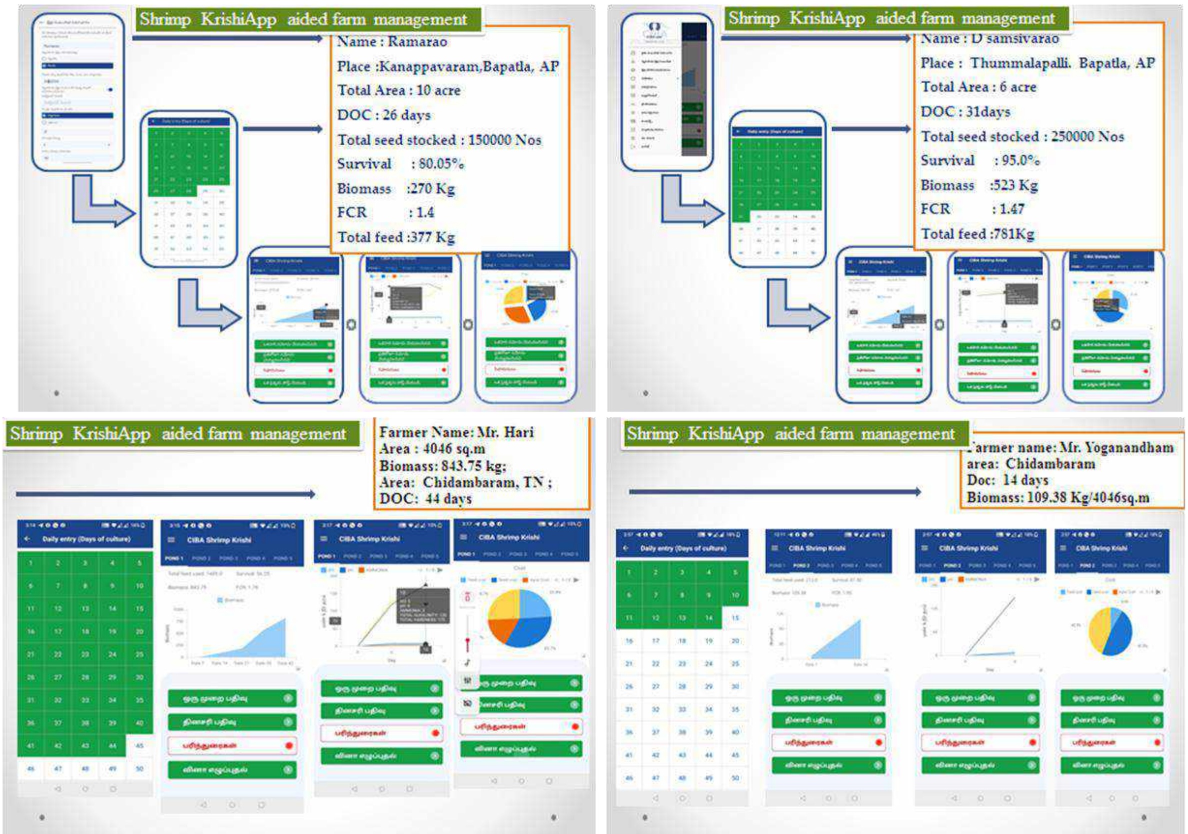
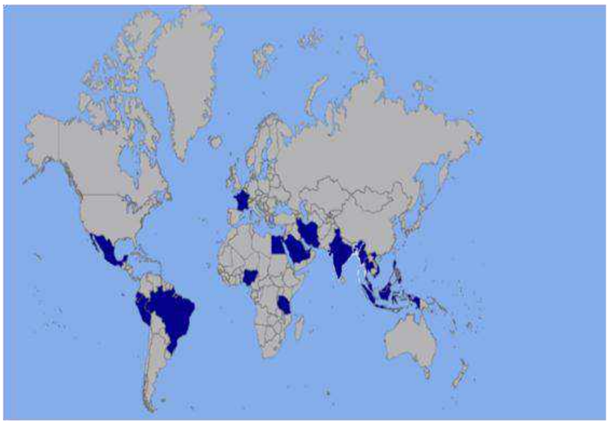


Fig-5. Successful adoption of CIBA Shrimp Krishi by the farmers



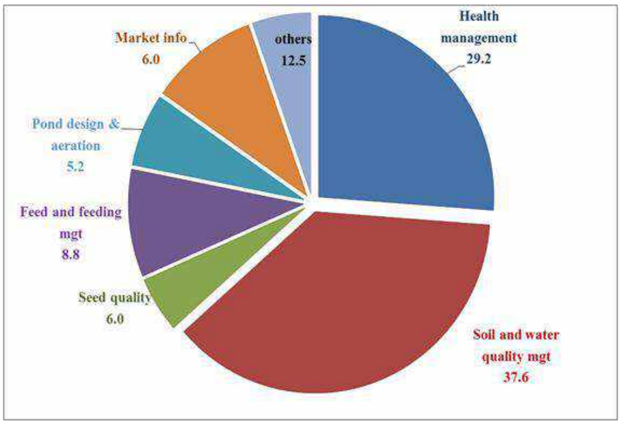


Fig -6. CIBA ShrimpApp users across countries

Fig-7. Subject matter wise queries received and answered through app

Likewise the initial feedback from the end-user farmers on Shrimp Krishi indicated that the app is highly useful for efficient shrimp farm management by acquiring, storing and sharing the data, helped in inputs optimization, tool for farm traceability & certification, very handy as the app is in regional languages, facilitated the farmer in crop planning and helped efficient decision making based on real-time data. There are suggestions like convert the app in windows platform, wider sensitization and training to the end users for effective use of the tool by the farming community. An evaluation study conducted among sample regular users indicated that the app aided in farm decision making and its design functionality and extension service function were perceived to be efficient.

Conclusion

Evaluations and field feedback have shown that CIBA mobile applications are an important contribution to the shrimp farming sector and found effective in disseminating the technology information to the end users. The farmers and extension workers perceived mobile application as a potential tool for knowledge improvement and real-time data based shrimp farm management. Apps enabled the bidirectional flow of information between the research institution and end users in getting field feedback through receiving and answering queries. Considering the all-pervading mobile connectivity, mobile application based technology advisories play a major role in minimizing the information communication gap in shrimp aquaculture and it may speed up and enhance the quality of the farm extension services. However, the efficiency of mobile application for extension services would depend on constant updating of the modules based on field requirements.

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Practicals

Collection, preservation and processing of samples for detection of shrimp pathogens by PCR

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Introduction

For investigating disease, moribund sample (infected animals those are about to die) are best samples for diagnosis. However, individuals from the same pond may also be collected fixed appropriately and provided to the diagnostic laboratory. Collection, storage and archiving of specimens and tissue samples are prerequisites for the successful acquisition of molecular data for any systematic study. This chapter reviews the important practical aspects of the sampling and storage: 1) selection of appropriate tissues for nucleic acid extraction 2) storage of freshly collected tissues in the field 3) transportation, long-term storage and archiving of tissue samples.The likelihood that this study will yield statistically significant results depends on the sample size. Sample sizes are directly dependent on the assumptions on the number of animals present in the pond or tank (table). Sample numbers should be adequate to understand the prevalence of disease at a given confidence limit

Sample sizes needed to detect at least one infected host in a population of a given size, at a given prevalence of infection.

Population size	**Prevalence (%)**						
	0.5	1.0	2.0	3.0	4.0	5.0	10.0
50	46	46	46	37	37	29	20
100	93	93	76	61	50	43	23
250	192	156	110	75	62	49	25
500	314	223	127	88	67	54	26
1000	448	256	136	92	69	55	27
2500	512	279	142	95	71	56	27
5000	562	288	145	96	71	57	27
10000	579	292	146	96	72	57	27
100000	594	296	147	97	72	57	27
1000000	596	297	147	97	72	57	27
>10000000	600	300	150	100	75	60	30

Sampling method for disease diagnosis

Sampling and fixation is one of the most crucial steps for precise shrimp disease diagnosis. Sampling must ensure an accurate representation of the health status of the population or individual. Sampling can be either lethal or nonlethal. Non-lethal sampling is usually carried out with brood stocks, where tip of the pleopod or faecal threads are used for PCR without sacrificing the animal. For lethal sampling, entire larvae or any tissue material such as hemolymph, gill, muscle, pleopod, lymphoid organ, hepatopancreas and eye stalk collected based on the type of viral pathogen needed for detection. Egg and larvae (~ 150 numbers of egg or larvae- nauplii to mysis) or ~10 PL depending on size/age) can be taken as whole and pooled sample to represent a mass. In case of late PL, it is preferred to cut the head and take abdominal portion to avoid PCR inhibitors. 3-6 diseased or moribund shrimps and an equal number of normal shrimps should be collected and packed separately.

The selection of particular tissue type is mandatory in the accurate diagnosis of viral infection. Because shrimp viral pathpgen infects particular cells and tissues of a host which support growth of a particular virus. Some viruses have a broad tissue tropism and can infect many types of cells and tissues. Other viruses may infect primarily a single tissue. For example White spot syndrome virus (WSSV) infects ectodermal and mesodermal origin tissues such as epidermis, gills, pleopod and hemolymph. But monodon baculovirus(MBV) infects only endodermal origin tissue hepatpancreas. The selected tissue of the organism should be relatively free of compounds potentially damaging to the nucleic acid or interfere with PCR. For example, Eye balls are known to contain PCR inhibitors.

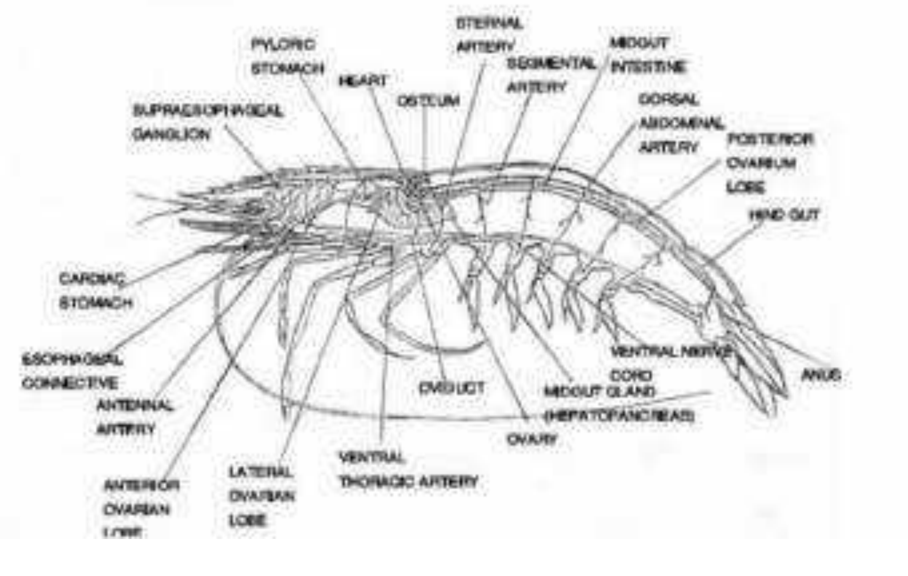

Fig. 1: Morphology of shrimp

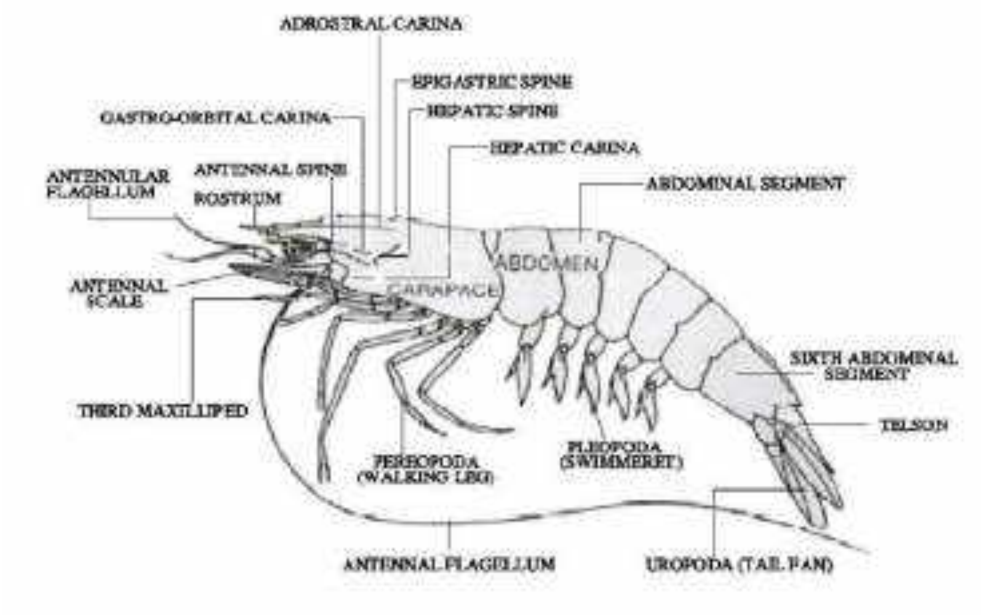

Fig. 2: Anatomy of shrimp

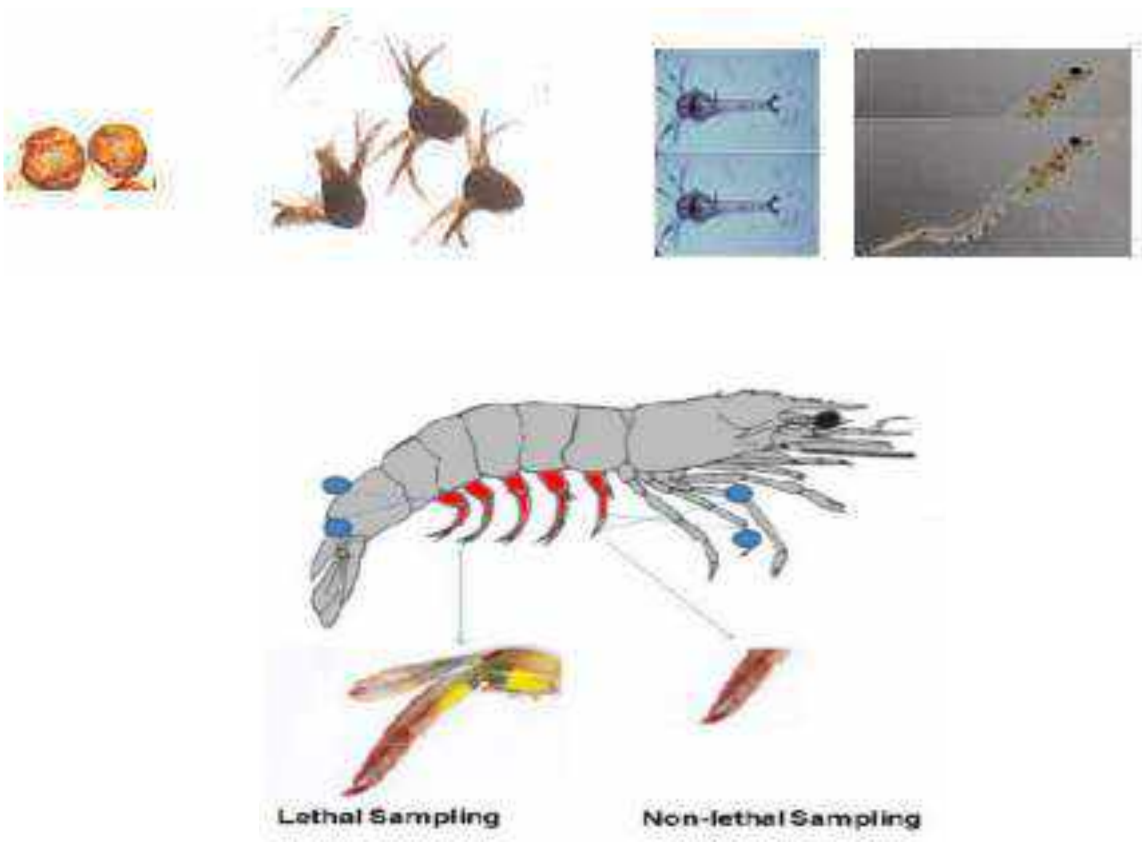

Fig. 3: Sampling of shrimp for disease diagnosis

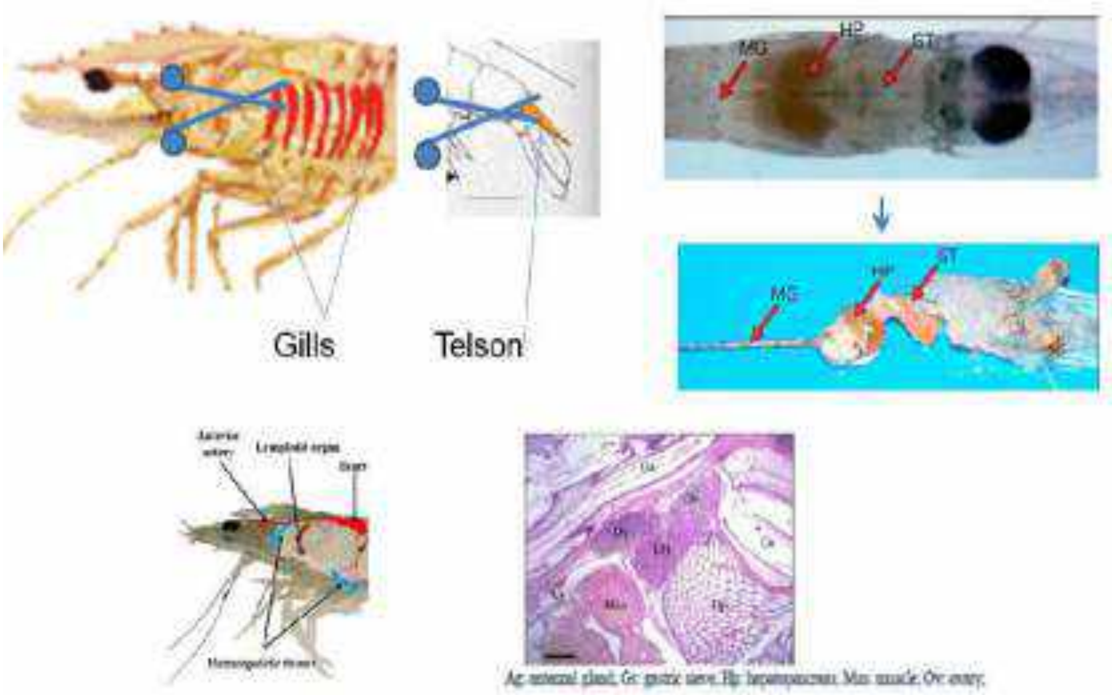

Fig. 4: Tissues of diagnostic importance

Target organs for detection of viruses (DNA & RNA), bacteria and Parasites infecting shrimp

Pathogen	Abbreviation	Target organs	Genome
DNA Viruses			
White spot syndrome virus	WSSV	larvae, pleopod, gill, hypodermis, hemocytes	dsDNA
Monodon baculovirus	MBV	larvae, hepatopancreas	dsDNA
Baculoviral midgut gland necrosis virus	BMNV	larvae, hepatopancreas	dsDNA
Baculovirus penaei	BP	larvae, hepatopancreas, anterior midgut	dsDNA
Spawner isolated mortality virus	SMV	larvae, hepatopancreas, midgut	ssDNA
Hepatopancreatic parvovirus	HPV	larvae, hepatopancreas	ssDNA
Infectious hypodermal haeamtopoietic necrosis virus	IHHNV	larvae, pleopod, gill, hypodermis, haematopoietic tissues, lymphoid organ	ssDNA

RNA Viruses			
Yellow head virus	YHV	larvae, gill, gut, gonads, pleopod, hemocytes, lympoid organ	(+)ssRNA
Taura syndrome virus	TSV	larvae, gill, gut, striated muscle, pleopod, hypodermis, lympoid organ	(+)ssRNA
Infectious myonecrosis virus	IMNV	larvae, skletal muscles, lympoid organ, hemocytes	(+)ssRNA
Mouriliyan virus	MoV	larvae, gill, lympoid organ, cuticular epithelium	(-) ssRNA
Gill associated virus	GAV	larvae, gill, gut, gonads, pleopod, hemocytes, lympoid organ	(+)ssRNA
Lymphoid organ vacuolization virus	LOVV	larvae, lympoid organ,	(+)ssRNA
Laem singh virus	LSNV	larvae, gills, lympoid organ, nervous tissues	ssRNA
PARASITE			
Enterocytozoon hepatopenaei	EHP	larvae, hepatopancreas	ds DNA
BACTERIA			
Necrotizing hepatopancreatitis	NHP	larvae, hepatopancreas	ds DNA
Acute hepatopancreatic necrosis disease	AHPND	larvae, hepatopancreas, bacterial culture from ds DNA ,gut tissue of live shrimp	ds DNA

Preservation of tissue

Fresh material from live animals consistently provides the highest yield and quality of nucleic acidfor amplification. The live animals or moribund animals can be frozen in dry ice and rapidly placed in the cold and away from light. The tissues should be packed in plastic cryotubes or Ziploc bags excluding as much air as possible to avoid cross contamination.

The tissue samples can be stored and transported in 95–100% ethanol at ambient temperature. The larger size or exoskeleton of the animal does not allow the penetration of ethanol of the tissue and causes degradation of the tissues. These samples should be injected with ethanol, dissected into smaller pieces to allow the ethanol to diffuse directly into the internal tissues. There should be about ten volumes of ethanol to one volume of sample for the proper preservation of the sample. Ethanol should be replaced after the initial fixation and periodically at a regular interval.

Long-term storage conditions should minimise variation in temperature. The animal tissues will remain indefinitely stable for extraction of nucleic acids at –70 to –80 °C.

This will allows the archiving of samples for reanalysis. There are also several commercial preservative available specifically to preserve nucleic acid in tissue.

Steps to avoid Contamination

The investigator should be aware of the importance of keeping their instruments, containers and reagents clean in order to prevent cross-contamination. The individual tissue samples should be stored in separate containers. The investigator should label and document all materials they collect with the details such as date of collection, collector, voucher number, etc with the permanent ink marker.

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Isolation and identification of bacteria from shrimp

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Sampling:

- *Hemolymph* – Draw aseptically from the heart or from the base of the periopod
- *From infected parts with necrosis/blisters* – wash the area with sterile saline to get rid of external contamination. With the help of a sterile swab, collect sample from the infected area
- *From internal organs* – Dissect out shrimp carefully to avoid any contamination from nearby organs. Sampling can be done from Hepatopancreas with an inoculating loop or swab. Similarly, other organs can be taken aseptically, homogenized with 1% TSB and proceed for culture

Culture:

- Sample can be put either on TCBS or ZMA plates (1 or 2 drops of hemolymph and spread, streak the swab on plates, inoculate 100 µl from the homogenized part of internal organ and then spread)
- Incubate at 30 ^{0}C for 18-24 hours for colonies to develop

Identification by Biochemical characterization of bacterial isolates:

Identification of bacterial isolates is done based on the colony characteristics, physiological and biochemical characteristics.

Amino Acid Decarboxylase Test

- Prepare Moeller's decarboxylase broth base with 1% arginine, Moeller's decarboxylase broth base with 1% ornithine and Moeller's decarboxylase broth base with 1% lysine and dispense in tubes before sterilizing.
- Inoculate freshly prepared bacterial culture into the above broth using sterile inoculation loop. Overlay with sterile mineral (paraffin) oil after inoculation.
- Incubate at 37^{o}C and observe for four days.
- Development of purple colour is positive, yellow colour represents negative result.

Salt Tolerance

- Prepare Tryptone water broth by adding 0.5 gm of Tryptone, 0.2 gm of yeast extract with 0%, 3%, 8% and 11% of NaCl to 100 ml of distilled water and transfer the prepared contents to each test tube and keep for sterilization.
- Pick single colony from TCBS plate and inoculate in sterile ZMA broth and incubate at 30 ºC for 24 hrs.
- Innoculatefreshly prepared bacterial culture into test tubes with different salt concentration andincubate at 37^{o}C for 24 hrs and observe the medium for turbidity.

Indole Production Test

- This test detects production of indole from tryptophan.
- Inoculate the tryptone water with bacterial culture and incubate for 24 hours.
- After incubation add 0.5 ml of Kovac's reagent.
- In positive cases a pink colour ring appears is positive, yellow ring indicate negative result.

Methyl Red

- This test is performed to identify bacteria which produce lactic, acetic or formic acid from glucose via mixed acid fermentation pathways.
- PrepareMRVP broth and inoculate with freshly prepared broth culture and incubate for 48-72 hrs
- Add a few drops of methyl red indicator solution.
- Development of strong red colour indicates positive reaction.

Voges Proskauer's Test (MRVP)

- This test detects the production of acetyl methyl carbinol as chief end product of glucose metabolism.
- PrepareMRVP broth and inoculate with freshly prepared broth culture and incubate for 48-72 hrs
- After incubation, add drops of alpha-naphthol and 40% sodium hydroxide along the walls of the test tubes carefully.
- Shake the test tubes at intervals to ensure maximum aeration. Appearance of red color indicates positive result for VP, whereas yellow color indicates negative result for VP.
- To above broth culture add Methyl red indicator

Fermentative Utilisation of Carbohydrates

- Prepare Phenol red broth base supplemented with 1% Salt and 1% Lactose, similarly prepare 1% Salicin, 1% Sucrose and 1% Mannitol, 1%Maltose and 1% Starch and dispense into tubes and sterilize.
- Inoculate the broth with freshly prepared bacterial culture and incubate at 37^{o}C and observe for colour change at 24, 48 and 72 hr. intervals.
- Appearance of pink colour indicates positive result and yellow colour indicate negative result.

Oxidase Test

- Place an oxidase disc in a clean glass slide and moisten with distilled water.
- Pick single colony and place on moistened surface of oxidase disc.
- Observe for colour change immediately, purple colour as positive and yellow colour as negative.

Citrate Test

- Prepare Simmon Citrate Agar slants and inoculate by streaking the slants using sterile inoculation loop with freshly prepared bacterial culture and incubate at 30 ºC for 24 to 48 hr and observe for growth and colour change.
- Colour change of slants to blue is positive and green colour slantrepresents negative.

Antibiotic sensitivity test:

- The organisms were grown for 24 hrs in ZMA broth and 0.1 ml of culture was uniformly spreaded on the ZMA plates.
- The antibiotic discs were gently pressed and placed at equal distance and the plates were incubated at 37 ºC for 24 hrs.
- The following nine antibiotic discs namely Ampicillin, Streptomycin, Neomycin, Tetracycline, Oxytetracycline, Ciprofloxacin, Chloromphenicol, were used and observed for the zone of inhibition.

Nitrate Reduction Test

- Nitrate broth is used to determine the ability of an organism to reduce nitrate (NO3) to nitrite (NO2) using the enzyme nitrate reductase. It also tests the ability of organisms to perform nitrification on nitrate and nitrite to produce molecular nitrogen.
- Prepare Nitrate broth and incubate after inoculation with bacterial culture
- After incubating the nitrate broth, add a dropper ful of sulfanilic acid and a-naphthylamine. If the organism has reduced nitrate to nitrite, the nitrites in the

medium will form a red-colored compound. Therefore, if the medium turns red after the addition of the nitrate reagents, it is considered a positive result for nitrate reduction. If the medium does not turn red after the addition of the reagents, it can mean that the organism was unable to reduce the nitrate. Therefore, another step is needed in the test. If the medium does not turn red after the addition of the nitrate reagents, add a small amount of powdered zinc. If the tube turns red after the addition of the zinc, it means that unreduced nitrate was present. Therefore, a red color on the second step is a negative result. If the medium does not turn red after the addition of the zinc powder, then the result is called a positive complete.

A typical chart is presented below for the identification. Several of such charts can be found in the published literature based on which, the bacterial can be identified.

	V. alginolyticus	*V. anguillarum*	*V. carchariae*	*V. cholerae*	*V. cincinnatiensis*	*V. damsela*	*V. fluvialis*	*V. furnissii*	*V. harveyi*	*V. metschnikovii*	*V. mimicus*	*V. parahaemolyticus*	*V. vulnificus*	*A. hydrophila*	*P. shigelloides*
Growth in TCBS	Y	Y	Y	Y	Y	G	Y	Y	Y/G	Y	G	G	G	Y	G
Oxidase	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Growth in :															
0% NaCl	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+
6% NaCl	+	+	+	-	+	V	+	+	+	+	-	+	+	+	-
ONPG	-	+	-	+	+	-	+	+	V	+	+	-	+	+	-
Voges-Proskauer	+	+	-	V	+	+	-	-	-	+	-	-	-	+	-
Lysine decarboxylase	+	-	+	+	+	V	-	-	+	+	+	+	-	V	+
Acid from:															
D-cellobiose	-	+	+	-	+	+	+	-	nd	-	-	V	+	+	-

[a] Y = yellow, G = green. V = variable. nd = not determined. + = positive and - = negative

Antibiotic sensitivity test:

- The organisms were grown for 24 hrs in ZMA broth and 0.1 ml of culture was uniformly spreaded on the ZMA plates.
- The antibiotic discs were gently pressed and placed at equal distance and the plates were incubated at 37 ºC for 24 hrs.
- The following nine antibiotic discs namely Ampicillin, Streptomycin, Neomycin, Tetracycline, Oxytetracycline, Ciprofloxacin, Chloromphenicol, were used and observed for the zone of inhibition.

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PCR protocol for detection of *Enterocytozoon hepatopenaei* infection in shrimp

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Sample collection

For PCR detection, fresh or ethanol-preserved samples of post larvae (PL) from hatchery or whole hepatopancreas in case of grow-out monitoring may be used. As the microsporidian spores are expelled out through feces, fresh fecal sample from precious or specific pathogen-free (SPF) broodstock may also be used as non-lethal sample for analysis.

DNA Extraction

- Take 20-30 mg of sample in 150 µl lysis buffer (50 mMTris pH 8.0- 606 mg, 1mM EDTA- 37.2 mg, 500 mMNaCl- 2.922 gm, 1% SDS – 1 gm make up to 100 ml with Proteinase K @ 10 µg/ml) and homogenize the sample with the micropestle.
- Add 350 µl of lysis buffer to the homogenate and mix well by vortexing.
- Incubate the sample at 95^{o}C in dry bath for 1 min.
- Cool the lysate to room temperature and centrifuge the sample at 12,000 rpm for 10 min.
- Collect 200 µl of supernatant into fresh tube with a micropipette and 500 µl of 70% ethanol and mix by invert mixing.
- Centrifuge the tube at 12,000 rpm for 10 min and decant the supernatant without disturbing the DNA pellet.
- Invert the tube on a clean dry tissue wipe and air dry the pellet.
- Reconstitute the DNA pellet in 200 µl of water or TE buffer (1 ml 1M Tris (pH 8.3) and 200 µl 0.5 M EDTA (pH 8.0) and adjust the volume to 100 ml of de-ionized water).
- Use 1 µl of this sample as DNA template for the PCR.

Quantification of DNA

The concentration of DNA should be determined by measuring the absorbance at 260 nm in a spectrophotometer. For quantification of DNA, take 10 µl of DNA and dilute in 990 µl of TE buffer. Take the O.D at 260 nm and the concentration of DNA to be calculated as follows.

1 OD of dsDNA at 260nm = 50 µg/ml

Therefore, DNA concentration (µg/ml) $= \dfrac{\text{O.D x Dilution factor x 50}}{1000}$

The concentration of DNA should be adjusted to 50 µg/µl with TE buffer for PCR analysis.The extracted DNA can be kept for long period at 4^{o}C.

Method : The PCR procedure followed as described by Jaroenlak *et al*. (2016)

PCR Primers details for the detection of *E. hepatopenaei*

Primers	Sequence	Amplicon Size
Spore wall protein		
SWP 1F	TTGCAGAGTGTTGTTAAGGGTTT	First step
SWP 1R	CACGATGTGTCTTTGCAATTTTC	514 bp
SWP 2F	TTGGCGGCACAATTCTCAAACA	Nested
SWP 2R	GCTGTTTGTCTCCAACTGTATTTGA	148 bp

Setting up the PCR Reaction

Once we choose the appropriate substrate DNA and PCR primer sequences, the PCR reaction can be set up as follows. Take care not to cross-contaminate the reagents, especially the templates and primers. Pipetting order in general; we may add water first and the enzyme last. For a large number of reactions, it is good practice to first set-up a master mix of the common reagents and then aliquot them, rather than to pipette the reagents separately for each individual tube. An example for a typical reaction of 25 µl set up is shown below:

Step 1 (First step PCR)

Reagents	**Vol (µl)**	**Vol (µl) (X) no. of reactions**
Water	9.5	
2x Master Mix (contains Buffer with $MgCl_2$, dNTPs, Taq DNA polymerase)	12.5	
Forward primer (10 µM)	1	
Reverse primer (10 µM)	1	
DNA template	1	
* Original Rxn. Vol. 25 µl	25 µl	

Step 2 (Nested PCR)

Reagents	**Vol (µl)**	**Vol (µl) (X) no. of reactions**
Water	9.5	
2x Master Mix (contains Buffer with $MgCl_2$, dNTPs, Taq DNA polymerase)	12.5	
Forward primer (10 µM)	1	
Reverse primer (10 µM)	1	
First PCR product (template)	1	
* Original Rxn. Vol. 25 µl	25 µl	

The reaction conditions for each step are provided above along with references

Observations and Documentation

After completion of the PCR, 1.6% agarose gel prepared either in 1x Tris-Acetate-EDTA buffer (1 litre 50x TAE–242 g Tris Base,55 ml Glacial Acetic acid and 37.2 g EDTA, pH 8) or 0.5 x Tris Boric acid EDTA buffer (1 litre 50x TBE – Tris base 540 g, Boric acid 275 g and EDTA 18.5 g, pH 8.0). Ethidium bromide is added to the molten agarose (0.5 µg/ml final concentration) when temperature reached 45-50^{o}C and then poured into the casting tray. Once the gels are solidified, it is submerged in the tank with the same buffer. A total volume of 5-10 µl amplified product is directly loaded in sample wells and 4 µl of the 100 bp DNA ladder loaded to the marker well in gel to verify the size of the amplified product. After loading, the tank is connected to a power pack and electrophoresis is carried out at voltage of 80-120. Continue the electrophoresis until the dye migrates to the appropriate distance in the gel and visualise under UV transilluminator.

Representative PCR gel electrophoresis

Fig. 1. Gel image of EHP spore wall protein PCR First step.

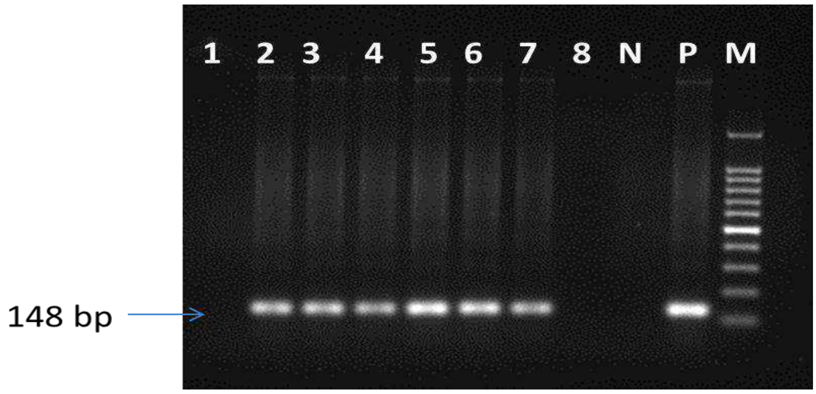

Fig. 2. Gel image of EHP spore wall protein PCR second step.

Bibliography

1. Jaroenlak, P., Sanguanrut, P., Williams, B.A., Stentiford, G.D., Flegel, T.W., Sritunyalucksana, K. and Itsathitphaisarn, O., 2016. A nested PCR assay to avoid false positive detection of the microsporidian *Enterocytozoon hepatopenaei* (EHP) in environmental samples in shrimp farms. *PLoS One*, 11, e0166320.

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